

VOLUME 37

DECEMBER 1959

NUMBER 6

# *Canadian Journal of Zoology*

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## Canadian Journal of Zoology

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Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

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## STUDIES ON DIPTEROUS PARASITES OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

### VII. *AGRIA AFFINIS* (FALL.) (DIPTERA: SARCOPHAGIDAE)<sup>1</sup>

H. C. COPPEL,<sup>2</sup> H. L. HOUSE, AND M. G. MAW

#### Abstract

*Agria affinis* (Fall.), a holarctic parasite of Lepidoptera, Orthoptera, and Hymenoptera and one of the more common of the native sarcophagid parasites of *Choristoneura fumiferana* (Clem.) in British Columbia, deposits first stage larvae on or near the late larval and pupal stages of the host. The larvae penetrate the host integument and complete their development inside the host, dropping to the ground to overwinter as puparia. Adults emerge the following spring. The species was reared in the laboratory continuously on pork liver. Mated females had a prelarviposition period of about 21 days and deposited larvae for up to 45 days. Larval development was completed in 5 to 8 days, and at  $21 \pm 1^\circ \text{C}$  and 60% R.H. the puparia formed within 24 hours. The adults emerged from puparia after 10 to 14 days if dormancy did not intervene. *A. affinis* is propagated continuously in the laboratory, as the stock now appears to have no significant pupal diapause. Among the important characters for identifying its immature stages are the forms of the buccopharyngeal apparatus and of the anterior and posterior spiracles.

#### Introduction

The sarcophagid parasite *Agria affinis* (Fall.) was recorded annually from 1944 to 1949 from collections of the spruce budworm, *Choristoneura fumiferana* (Clem.), made in British Columbia. It was always obtained in larger numbers in collections from Western Canada than from Eastern Canada. It was listed by Coppel (4) and by Wilkes, Coppel, and Mathers (26) respectively, as third and fourth in importance in British Columbia among the dipterous parasites of the spruce budworm and fourth and seventh among all parasites of that species. It was responsible for 50.9% of the total parasitism at Fountain Valley, B.C., in 1947.

In the Belleville laboratory an attempt was made to propagate *A. affinis* obtained from the West for release in Eastern Canada. *A. affinis* originally obtained from British Columbia was reared on *C. fumiferana* and *Pieris*

<sup>1</sup>Manuscript received June 22, 1959.

<sup>2</sup>Contribution from Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario.

<sup>3</sup>Entomology Laboratory, Belleville, Ontario; now in Department of Entomology, University of Wisconsin, Madison, Wisconsin.

*rapae* (L.), but now is propagated mainly on pork liver. More than 50,000 adults were released in Manitoba, Ontario, Quebec, New Brunswick, and Newfoundland.

This paper on *A. affinis* includes systematic position, distribution, hosts, rearing methods, descriptive features, life history, and habits, especially of laboratory-reared specimens, and some field observations on parasitism in different areas of Canada.

### Systematic Position, Distribution, and Hosts

*Agria affinis* was originally described in 1816 as *Musca affinis* by Fallen (6). Robineau-Desvoidy (21), in 1830, erected the genus *Agria* for six new species, one of which, *Agria punctata*, was later designated as the type by Townsend (24) in 1916. Macquart (19) and early authors considered *A. punctata* a synonym of *M. affinis*. In 1888, Wachtl (25) placed *M. affinis* in the genus *Sarcophaga*. Kramer (18), in 1908, established the genus *Pseudosarcophaga* on the basis of the morphology of the male genitalia. He included *Sarcophaga affinis* Fallen and two other species. In 1928, Enderlein (5) designated *affinis* as the type of *Pseudosarcophaga*, making *Pseudosarcophaga* Kramer a synonym of *Agria* Robineau-Desvoidy. Though the name *Pseudosarcophaga affinis* has been used extensively in Canada during the past 10 years, an exhaustive search and analysis of the literature by Mr. G. Shewell (personal communication), Entomology Research Branch, Canada Department of Agriculture, Ottawa, left very little doubt that this species is correctly named *Agria affinis*.

*A. affinis* (Fall.) is holarctic in distribution. It was listed from Austria, Poland, Czechoslovakia, Finland, Sweden, Yugoslavia, Italy, Germany, Spain, Russia, North Africa, and Canada by Thompson (23), from Ireland by Beirne (3), and from the United States by Aldrich (1). Specific collection areas in Canada investigated by staff of the Belleville laboratory are listed in Table III.

According to Thompson (23) the hosts are of the families Pieridae, Lasio-campidae, Yponomeutidae, Lymantridae, Nymphalidae, and Tortricidae (Lepidoptera), and Acrididae (Orthoptera) and Tenthredinidae (Hymenoptera).

### Materials and Methods

*A. affinis* may be reared in the laboratory at  $21 \pm 1^\circ$  C and 60% R.H. If it is confined with its natural hosts, parasitism readily occurs and adults are obtained after incubation at room temperature. Host supplies limit this method. The parasite may be propagated continuously on several kinds of unnatural food materials. Early methods on a liver and fish medium were described by House (9) and House and Traer (17). Since then many modifications in technique have been developed, including special equipment (House and Barlow (16)) and use of chemically defined diets and axenic cultural methods (House (10)) for nutritional determinations (House (11-13),

House and Barlow (14, 15)). At first, laboratory propagation was curtailed seasonally because diapause interrupted pupal development for several months. The diapause seems to have been now largely eliminated, probably because successive generations of breeding stock were obtained mainly from individuals that underwent no diapause.

Large numbers may be propagated for biological control programs with little manipulation of the insect or equipment. The breeding cage (Fig. 1), about 12 in. by 12 in. by 18 in. in size, is of a wooden frame enamelled white for sanitation and covered on three sides and the top with plastic mesh; the front is a sheet of clear plastic with an opening for servicing the cage, and slides upwards in saw kerfs for removal; the floor is wood painted black and covered with a sheet of clear plastic, which slides forward in saw kerfs and can be easily removed without opening the cage. About 1000 flies are confined in this cage and mating and reproduction occur spontaneously. No further handling is necessary except to feed and remove larvae. They are fed about a 10% aqueous solution of honey soaked into a coil of dental cotton in a small dish, though water and cubes of sucrose seem adequate and do not sour as does the honey solution. Fresh pork liver is placed on a waxed paper mat on the plastic floor covering. The flies are attracted to the liver and feed on its juices, supplementing their diet of sugars and water. Active larvae are deposited on or near the liver. Every day or so the plastic floor covering is removed and the larvae collected from the liver or taken with a small brush from the plastic sheet held to the light.

A thousand or more larvae are reared on about half a pound of sliced fresh liver (cf. 17) in a covered ventilated dish (Fig. 2). Here they feed to satiation, apparently unaffected by putrefaction of the liver, though stale liver or liver that has been frozen causes high mortality among young larvae. Usually the larvae complete their feeding a day or so after reaching the third instar and move away from the food to a suitable pupation site. When this occurs they are transferred with a spoon to a box of dry sawdust. Later the sawdust is sifted through a screen to collect the puparia.

A thousand or more puparia are put into a wooden container (Fig. 3). It is about 4 in. by 4 in. by 2½ in. in size and enamelled white on the outside and black on the inside; one side (A) is elongated and has a short tubular sleeve (B) projecting from near the top center; a perforated plastic top (C) slides in saw kerfs. The container is attached to the opening in the plastic front of the breeding cage by inserting the sleeve and hooking its saw kerf (D) over the edge of the plastic, and is supported in position by the elongated side of the container bearing against the front of the breeding cage. As the flies emerge they escape through the sleeve opening into the breeding cage, mate, and subsequently deposit larvae on and near the liver provided for them. Though this method is especially suited to propagation of large numbers, where time and care must be minimized, it probably is not as efficient or suitable for certain purposes as some other techniques.

When detailed records on *A. affinis* were required, rearing techniques and equipment as described for *Sarcophaga aldrichi* Park. (Arthur and Coppel

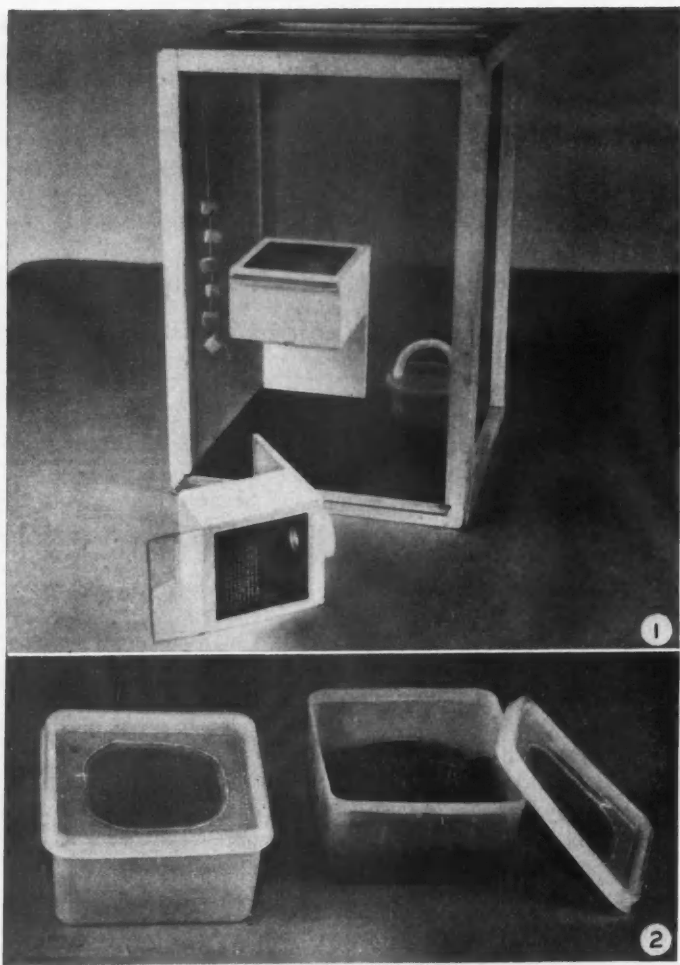


FIG. 1. Breeding cage with removable plastic floor covering, feeding dish, and sugar cubes. Two puparia containers are shown, one attached in position on cage.

FIG. 2. Ventilated plastic dish used to rear larvae on liver.

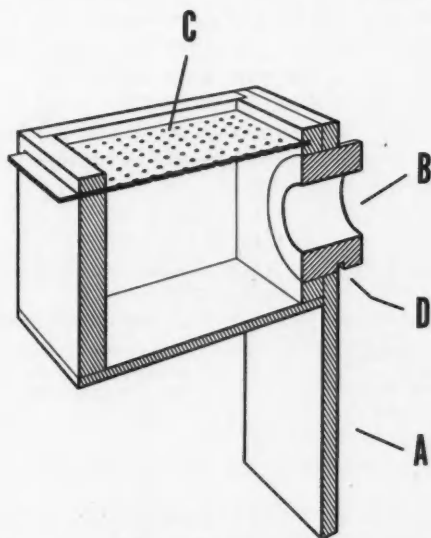


FIG. 3. Puparia container, in longitudinal sectional view; A is an elongated side with a tubular sleeve (B), C is a removable, perforated plastic top. B inserted into the opening in the front of the breeding cage hooks in place by the saw kerf (D).

(2)) were used, except that the larviposition cages had a removable plastic floor covering. Adult emergence was hastened and usually increased when puparia were exposed to sunlight and it was not necessary to spray the puparia with water.

Sometimes fully fed mature larvae linger on the food for many days and must be removed if they are to pupate readily. Repeated tests involving 400 such larvae showed that concentrated glacial acetic acid sprayed on the food caused the larvae to leave and pupate normally.

### Life History

#### *Larva*

*A. affinis* is viviparous. First instar larvae molt 25 to 27 hours after deposition and the second molt occurs 51 to 56 hours later. Third instar larvae feed for a day or so and increase slightly in size. Optimal postlarval development depends on the nourishment of the third instar larvae. When they are not allowed food, few pupate normally; those insufficiently fed produce small puparia and flies; those fed to satiation produce the largest puparia; and those fed for about 6 days usually pupate most readily. Thus, fully fed, mature larvae are obtained in 5 to 8 days when reared by the above-described laboratory techniques.

#### *Pupa*

Puparia may be formed by fully fed mature larvae in damp sawdust within 24 hours, though the majority require up to 3 days. A few larvae

may remain for as long as a week, but these usually form misshapen puparia. The pupal period lasts 10 to 14 days and the flies emerge then unless diapause occurs. If diapause occurs, the puparia must be held for about 6 months at 1° C in moist sawdust, and incubated then for about 10 days at 21° C to complete development. The percentage females for 3 years was  $48.6 \pm 1.5$ . Cold storage longer than 10 months results in high pupal mortality and a higher percentage of females (Table I).

TABLE I  
Effects of long storage of *A. affinis* puparia at 1° C on emergence  
and sex ratio

| Storage period,<br>months | No. of<br>puparia | Adult<br>emergence, % | Percentage<br>of females |
|---------------------------|-------------------|-----------------------|--------------------------|
| 6                         | 1737              | 66.2                  | 46.0                     |
| 7                         | 2034              | 74.4                  | 48.0                     |
| 8                         | 4142              | 63.3                  | 51.1                     |
| 9                         | 3361              | 66.5                  | 48.5                     |
| 10                        | 856               | 88.9                  | 53.2                     |
| 12                        | 680               | 27.2                  | 54.1                     |
| 15                        | 1069              | 14.3                  | 63.4                     |

Diapause occurs when the pupa is cream in color, the body divisions distinct, and the appendages clearly outlined. The first obvious indication of resumed development is the appearance of rust-colored pigment in the compound eyes.

*A. affinis* presumably has usually only one generation a year in the field, for, when laboratory propagation was first attempted in 1947, of 102 larvae laid by females obtained from western collections of *C. fumiferana* and reared on a liver and fish medium (17) all formed puparia and remained in diapause. However, by 1949, records on over 17,000 puparia showed that during 12 months approximately 88% of the puparia entered diapause and that seasonal changes occurred in the percentage entering diapause, as follows: January to March, 99.1; April to June, 76.0; July to September, 75.9; and October to December, 100.

#### Adult

The adult emerges 10 to 14 days after the puparium is formed; the male usually emerges 1 or 2 days before the female. Consequently development from newly deposited larva to adult may take up to 28 days. Longevity of the female averages more than 40 days, but the male does not live as long.

Matings usually occur when the males are 5 to 7 days old and the females are newly emerged. However, matings may occur when both sexes are a few hours old, and some females mate for the first time when 10 to 14 days old.

From three generations of about 100 females each, the average prelarviposition period was 21 days, with a range of 7 to 40 days.

Larviposition of 95 mated females reached a maximum about 21 days after mating and decreased gradually to zero at about 45 days (Fig. 4).

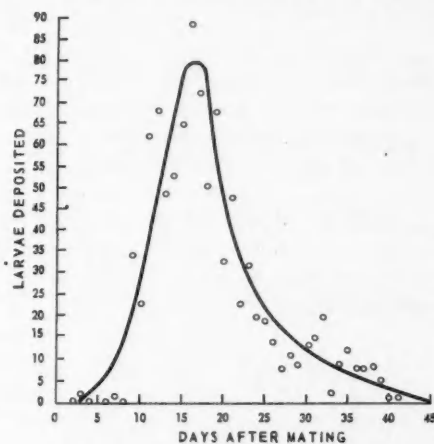
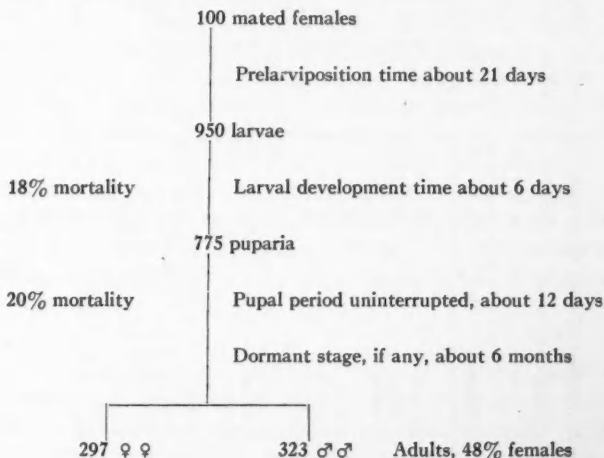


FIG. 4. Number of larvae deposited by 95 females each day after mating.

The average number of progeny per 100 females (600 mated females used), developmental times, and survival are summarized as follows:



#### Descriptions of Stages

##### Adult

An adequate description of both the male and the female was given by Aldrich (1).

##### Egg

Eggs, which are laid occasionally, especially by young females, are nonviable and, except for their small size, 1.5 mm long, are similar to those of *S. aldrichi* as described by Arthur and Coppel (2).



*First Instar Larva*

The larvae of *A. affinis* are typically sarcophagid in shape. One-day-old larvae are approximately 2.27 mm long and 0.34 mm wide. The internal organs, tracheal system, and cephaloskeleton are visible through the semi-transparent integument, which is armed with irregular bands of spines. The arrangement of these spines is shown in Table II. The spines of the anterior rows in the bands are usually scalelike and bear one or two heavily pigmented projections, whereas those of the two or three remaining rows are short and spearhead in shape. The spines become progressively smaller in the rows toward the posterior portion of each segment. The first instar larva is characterized by short, strong setae on the ventral and ventrolateral borders of the abdominal segments.

The pseudocephalon is conical and carries an antennomaxillary complex on each of its anterolateral surfaces. Each complex includes two large sensory organs and one small one. The anterior organ is composed of three segments, including a broad base. Behind this organ there is a fleshy lobe with several small papillae, each surrounded by concentric wrinkles. The smaller organ lies at the base of the lobe.

Hall's (8) nomenclature is used to describe the buccopharyngeal apparatus, unless otherwise stated. The apparatus (Figs. 5 and 6) is well developed and is articulated. It is approximately 0.35 mm long and is strongly arched. Lateral hooks lie on either side of the labial sclerite and each is attached to the lateral sclerites by a thin, pigmented band. The dental sclerite is small and is situated behind the lateral sclerites. The hypostomal arch lies below the point of articulation between the labial sclerite and the pharyngeal sclerites.

Respiration is metapneustic. There are two spiracles, situated in a deep cavity on the posterodorsal area of the last segment. Each (Fig. 7) consists of two sections united side by side, with their felt chambers merging to form a common attachment with the trachea. The spiracles are separated by a distance equal to their diameter. The spiracular cavity is surrounded by six papillae.

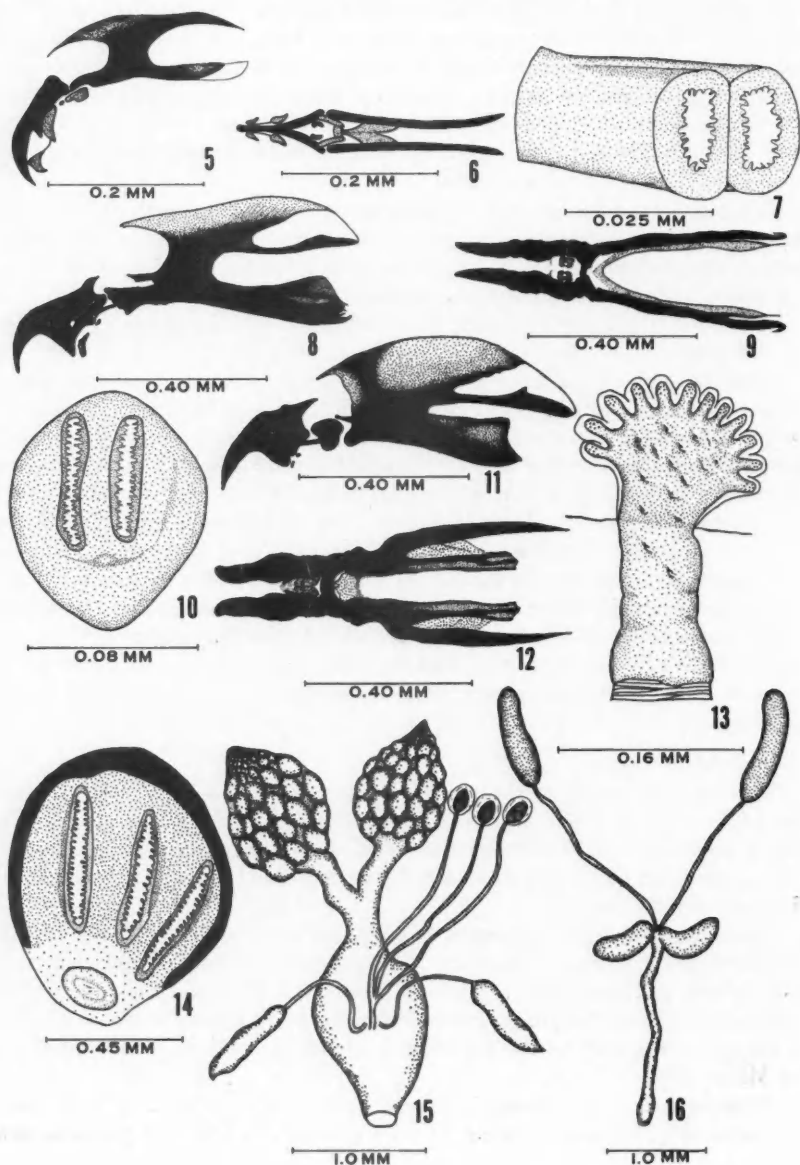
*Second Instar Larva*

The average length of the second instar larva is 4.0 mm, with a range of 2.4 to 6.5 mm. The armature consists of pigmented spines on the first three segments and semitransparent spines on the remainder (Table II). The antennomaxillary complex is similar to that found in the first instar but it is larger and flatter.

The buccopharyngeal apparatus (Figs. 8 and 9) of this instar can be readily distinguished from that of the first. The mandibular hooks, or labial sclerites, are paired and each has two small foramens. A moderately sclerotized rod projects from the pharyngeal sclerite dorsad to the hypostomal sclerite. This rod corresponds to the parastomal sclerite of Hall (8), or the atrial rod of Miller (20).

Respiration of the second instar larva is amphipneustic. The anterior spiracles (Fig. 13) are light-buff in color and project from the posterolateral

aspect of the second body segment. Each spiracle is covered with shallow pits and has 10 to 12 fleshy lobes, each with at least 12 orifices. The posterior spiracles (Fig. 10) are paired and light-buff in color and each has two sub-parallel slits. The spiracular plates are separated by a distance equal to the width of one plate, and the felt chambers are as wide as long.



### *Third Instar Larva*

The third instar larva is more robust than those of the preceding instars. Its length averages 9.2 mm and ranges from 6.0 to 13.4 mm. The cuticular spines are arranged in more regular rows than in the preceding instars and the pigmentation is similar to that of the second instar. Fusiform patches of spines, as recorded for *S. aldrichi* (2), occur on the posterior borders of the abdominal segments.

The buccopharyngeal apparatus (Figs. 11 and 12) is larger and appears more robust than that in the second instar larva. There are no foramens in the mandibular hook. There are, however, articulation points between the anterior, intermediate, and posterior parts of the apparatus.

The respiratory system in the third instar is like that of the second. The anterior spiracles are similar to those in the second instar, and the posterior spiracles (Fig. 14) are small and lightly pigmented. The peritreme is incomplete below the slits, and the posterior cavity is bordered by five pairs of fleshy protuberances.

### *The Puparium*

The puparium was illustrated by Greene (7) and Ross (22) and described by Greene (7).

## Reproductive Systems

The internal reproductive system of the female (Fig. 15) is typical of sarcophagids and is somewhat similar to that of *S. aldrichi* (2). The exact number of ovarioles is unknown, as they were poorly differentiated in the specimens examined; however, the number is small. The uterus and ovaries are surrounded by an extensive network of tracheoles.

The internal reproductive system of the male (Fig. 16) is characterized by the darkly pigmented and elongated testes. The vasa deferentia and ejaculatory duct are approximately equal in length. The accessory glands are unpigmented and nearly sessile. The ejaculatory duct is slightly enlarged near its posterior end. No ejaculatory pump was observed in the specimens examined.

## Habits

### *Laboratory Observations*

Mature larvae are negatively phototropic after they have ceased to feed; darkly shaded areas seemed as attractive as complete darkness. This response did not change with age after the larvae had fed. Larvae pupated in dry sawdust satisfactorily but more readily in a moist medium, such as peat moss or sawdust, and not in dry vermiculite.

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FIGS. 5-16. *Agria affinis* (Fall.). 5. Buccopharyngeal apparatus of first instar larva, lateral view. 6. Buccopharyngeal apparatus of first instar larva, dorsal view. 7. Felt chamber and posterior spiracle, first instar larva. 8. Buccopharyngeal apparatus of second instar larva, lateral view. 9. Buccopharyngeal apparatus of second instar larva, dorsal view. 10. Posterior spiracle of second instar larva. 11. Buccopharyngeal apparatus of third instar larva, lateral view. 12. Buccopharyngeal apparatus of third instar larva, dorsal view. 13. Anterior spiracle of second instar larva. 14. Posterior spiracle, third instar larva. 15. Internal reproductive system of female. 16. Internal reproductive system of male.

House and Traer (17) stated that puparia were formed by 38% of 759 larvae reared on the prepupae of *C. fumiferana* and by 88% of 13,000 larvae reared on liver and salmon.

There is no consistent pattern of behavior before mating; any movement of a fly may attract others. Usually the male flies or walks quickly and pounces upon a standing female with no preliminary courtship and very little struggle. Mating may also occur during flight, whereupon the pair falls to the floor of the cage. In any event the male stands on his metathoracic legs, grasps the female over the wings with his mesothoracic legs, and either waves his prothoracic legs in the air or rests them upon the female's dorsal metathoracic area. Direct sunlight and air currents appear to induce the mating response. Fifty copulations that resulted in larviposition varied from 64 to 187 seconds in duration and averaged 124. Multiple matings sometimes occur but their effects were not determined. Males frequently attempt coitus with other males, and two were observed together for as long as 2½ hours. They also attempt coitus with dead flies.

Females observed stalking mature spruce budworm larvae walked toward the host with quick, darting motions. Reflex movements of the host sometimes repel attacks by the parasite. Parasite larvae sometimes are found on the feeding tunnel spun and occupied by the host and these apparently gain entry into the host. As a female walks slowly over a surface, such as a cage floor, she extends the posterior end of her abdomen, curves it slightly forward, and quickly extrudes larvae, often singly or up to 10 in rapid succession. Females favor crevasses and depressions as larviposition sites. A small number of larvae are deposited for the first 4 or 5 days of larviposition, after which deposition is intermittent with no definite pattern. Records on three generations of mated females showed considerable variation:

|                                     |    |     |     |
|-------------------------------------|----|-----|-----|
| Number of females                   | 95 | 100 | 100 |
| Maximum number of larvae per female | 53 | 25  | 10  |
| Average number of larvae per female | 17 | 1.6 | 3   |
| Fertile females, %                  | 80 | 22  | 11  |

There was no appreciable difference in larviposition rates between females confined singly and together. For example, 47 fertile females caged singly averaged nine larvae and 41 females caged together averaged nine larvae, though three additional larvae per fly were obtained by postmortem dissection in the latter.

There are indications that the number of larvae per female may be greater than these larviposition records show, as dissection of large numbers of gravid females usually revealed 20 to 40 larvae in the uterus of each; as many as 56 were dissected from one. Larvae often emerge from their chorions within a dead fly and escape. It is not known whether death of the fly results from the actions of the larvae or from other causes. Ten per cent or more of the females die during the prelarviposition period.

#### *Field Observations*

Adults of *A. affinis* from overwintered puparia appear in the field in British Columbia from mid-June to mid-July, when *C. fumiferana* larvae are maturing

and the pupae forming. Mature larvae of *A. affinis* sometimes emerge from the host larvae or prepupae, but they emerge most frequently from host pupae. Though budworm pupae containing two small *A. affinis* larvae were sometimes observed, never more than one larva completed development in a host. Beirne's (3) study in Ireland and his reference to Servadei's work in Italy showed that the larvae of *A. affinis* were predaceous rather than parasitic, one individual killing 8 pupae of *Yponomeuta* sp. in 3 days and another killing 50 in 14 days. Hyperparasitism of *A. affinis* was rare. *Nasonia vitripennis* (Wlk.) (Hymenoptera:Pteromalidae) emerged from a few field-collected puparia in British Columbia. A white-footed mouse, *Peromyscus* sp., was the only vertebrate predator observed to eat the contents of an *A. affinis* puparium.

#### *Incidence of Parasitism*

Most of the data on abundance of *A. affinis* were obtained from large collections of *C. fumiferana* made annually in British Columbia from 1944 to 1949. Other data are from smaller collections made sporadically in Eastern Canada. In the main collection areas of British Columbia the peak years for parasitism by *A. affinis* were in 1947 and 1948. Though the percentage parasitism varied with localities and years, *A. affinis* was always among the most numerous of the parasites of the spruce budworm. A summary of data is shown in Table III.

TABLE III

Percentage parasitism of *Choristoneura fumiferana* by *Agria affinis* in various localities in Canada, 1944-1949\*

| Locality                | 1944 | 1945 | 1946 | 1947       | 1948       | 1949       |
|-------------------------|------|------|------|------------|------------|------------|
| Mt. McLean, B.C.        | 3.1  | 0.8  | 2.0  | 1.3 (3.7)  | 0.46 (1.9) | 1.4 (8.6)  |
| Texas Creek, B.C.       | —    | —    | 1.0  | 1.4 (40.9) | 6.27       | —          |
| Fountain Valley, B.C.   | —    | —    | 6.1  | 6.1 (50.9) | 3.6 (37.8) | 3.7 (50.0) |
| Mission Mountain, B.C.  | 1.6  | 0.2  | —    | —          | —          | —          |
| McGillivray Falls, B.C. | 0.1  | —    | —    | —          | —          | —          |
| Maniwaki, Que.          | —    | —    | —    | 0.1 (1.3)  | 0.2 (1.3)  | 1.8 (4.7)  |
| Forbes Depot, Que.      | —    | —    | —    | —          | 0.6 (4.3)  | —          |
| Island Falls, Ont.      | —    | —    | —    | 2.7 (5.1)  | —          | —          |
| Bowring Park, Nfld.†    | —    | —    | —    | 0.0 (0.2)  | —          | —          |

\*In parentheses percentage of the total parasitism of *C. fumiferana* by all species that were caused by *A. affinis*.

†Though *A. affinis* was released in the area in 1946 it is not known whether it was previously present there.

Sample collections of the budworm at 7-day intervals from the Mt. McLean area showed that parasitism by *A. affinis* was slightly greater than that for the general collections in the area. Percentage parasitism at the lower limit of infestation (1000-ft altitude) was 6.2 in 1948; at the central point of infestation (2000-ft altitude) it was 7.8 in 1947 and 3.3 in 1948; at the upper limit of infestation (3000-ft altitude) it was 10.9 in 1947 and 3.1 in 1948. A preliminary investigation showed that *A. affinis* was responsible for 2.6% parasitism of all budworms collected from Douglas fir, 0.6 from Engelmann spruce, 0.1 from Alpine fir, and 0.0 from juniper.

## References

1. ALDRICH, J. M. *Sarcophaga* and allies in North America. Thomas Say Foundation, Entomological Society of America, Lafayette, Ind. 1916.
2. ARTHUR, A. P. and COPPEL, H. C. Studies on dipterous parasites of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). I. *Sarcophaga aldrichi* Parker (Diptera: Sarcophagidae). Can. J. Zool. **31**, 374-391 (1953).
3. BEIRNE, B. P. The biology and control of the small ermine moths (*Hyponomeuta* spp.) in Ireland. Econ. Proc. Roy. Dublin Soc. **3**, 191-220 (1943).
4. COPPEL, H. C. The role of parasitoids and predators in the control of the spruce budworm (*Archips fumiferana* (Clem.)) in British Columbia. Ph. D. thesis. New York State College of Forestry. 1949.
5. ENDERLEIN, J. Klassifikation der Sarcophagiden. Arch. Klass. Phylog. Ent. **1**, 53 (1928).
6. FALLEN, C. F. Beskrifning öfver de i Sverige funne flugarter, som kunna föras till släktet *Musca*. Vetensk. Akad. Handl. 226-254 (1816).
7. GREENE, C. T. The puparia and larvae of sarcophagid flies. Proc. U.S. Natl. Museum, **66**, 29 (1925).
8. HALL, D. G. The blowflies of North America. Monumental Printing Co., Baltimore, Md. 1947.
9. HOUSE, H. L. Notes on the laboratory propagation of *Pseudosarcophaga affinis* (Fall.), a sarcophagid parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). Rept. Quebec Soc. Protect. Plants, 31st (1948-49), 134-137 (1951).
10. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). I. A chemically defined medium and aseptic-culture technique. Can. J. Zool. **32**, 331-341 (1954).
11. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). II. Effects of eleven vitamins on growth. Can. J. Zool. **32**, 342-350 (1954).
12. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). III. Effects of nineteen amino acids on growth. Can. J. Zool. **32**, 351-357 (1954).
13. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). IV. Effects of ribonucleic acid, glutathione, dextrose, a salt mixture, cholesterol, and fats. Can. J. Zool. **32**, 358-365 (1954).
14. HOUSE, H. L. and BARLOW, J. S. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). V. Effects of various concentrations of the amino acid mixture, dextrose, potassium ion, the salt mixture, and lard on growth and development; and a substitute for lard. Can. J. Zool. **34**, 182-189 (1956).
15. HOUSE, H. L. and BARLOW, J. S. Effects of nucleic acids on larval growth, pupation and adult emergence of *Pseudosarcophaga affinis* (Fall.). Nature, **180**, 44 (1957).
16. HOUSE, H. L. and BARLOW, J. S. Equipment for rearing small numbers of *Pseudosarcophaga affinis* (Fall.) (Diptera: Sarcophagidae) for experimental purposes. Can. Entomologist, **89**, 145-150 (1957).
17. HOUSE, H. L. and TRAER, M. G. An artificial food for rearing *Pseudosarcophaga affinis* (Fall.), a parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). Ann. Rept. Entomol. Soc. Ontario, **79** (1948), 50-53 (1949).
18. KRAMER, H. *Sarcophaga affinis* Fll. und Verwandte. Ent. Wochenbl. **25**, 200-201 (1908).
19. MACQUART, J. Insectes dipteres du nord de la France. Mém. soc. sci. agric. arts Lille, **5**, 182 (1834).
20. MILLER, D. The bucco-pharyngeal mechanism of a blow-fly larva (*Calliphora quadrimaculata* Swed.). Parasitology, **24**, 491-499 (1932).
21. ROBINEAU-DESVOIDY, J. B. Essai sur les Myodaires. Mém. acad. sci. inst. France, **2**, 376 (1830).
22. ROSS, D. A. Key to the puparia of dipterous parasites of *Choristoneura fumiferana* (Clem.). Can. Entomologist, **84**, 108-112 (1952).
23. THOMPSON, W. R. A catalogue of the parasites and predators of insect pests. Section 2. Part 1. Hosts of the Coleoptera and Diptera. Commonwealth Institute of Biological Control, Ottawa, Ont., Canada. 1951.
24. TOWNSEND, C. H. T. Designation of muscoid genotypes, with new genera and species. Insec. Inscit. Menstr. **4**, 6 (1916).
25. WACHTL, F. A. Biologische und synonymische Notizen über *Sarcophaga affinis* Fallen. Wien. Ent. Z. **7**, 277-278 (1888).
26. WILKES, A., COPPEL, H. C., and MATHERS, W. G. Notes on the insect parasites of the spruce budworm, *Choristoneura fumiferana* (Clem.) in British Columbia. Can. Entomologist, **80**, 138-155 (1948).

## A STUDY OF THE MUSCULATURE OF THE BLACK WIDOW SPIDER, *LATRODECTUS MACTANS* (FABR.)<sup>1</sup>

W. F. WHITEHEAD AND J. G. REMPEL

### Abstract

By means of serial sections and dissection, 537 individual muscles were found in the adult female *Latrodectus mactans*, and 533 in the male. The absence of muscles in the male palpal pretarsus represents the only substantial difference found between the muscular systems of the two sexes. The musculature corresponds generally to that described for other species of spiders. Among muscles found in the black widow spider, but not described in other spiders, are those of the coxal gland, the colulus, the ampullate silk duct, and the mid-gut, and some of the muscles of the pedicel, abdomen, and abdominal appendages. The muscles designated "abdominal sac" by various authors are considered to be vestiges of a laterally placed dorsoventral musculature originally joining tergites and sternites. The nature of the intrinsic musculature of the palpi supports the belief that these appendages represent modified legs. Homologies are proposed between the muscles of the pedicel and those of the abdomen, between the extrinsic muscles of the spinnerets and those of the coxae of the legs, and between the intrinsic muscles of the lateral spinnerets and those acting on the trochanter of the legs.

### Introduction

The black widow spider has acquired an infamy in both scientific and fictional literature. The venomous nature of the adult female has led to many studies of the symptoms, treatment, and prevention of her bite, but anatomical and physiological research not related directly to her toxicity has been neglected. In describing the musculature of the adult, an attempt has been made to furnish a basis for such research, and at the same time, to make some contribution to the knowledge of araneid musculature, a field which has been of value in determining segmental homologies and phylogenetic relationships among spiders.

The musculature of spiders received some of its earliest attention in the nineteenth century, in the publications of Treviranus (37), Wasmann (38), Frey and Leuckart (12), and Siebold (34). It was Kessler (16), however, in 1849, who first gave an accurate description of the general arrangement of muscles, and prepared the way for such nineteenth century workers as Boettcher (3), Leydig (20), Blanchard (2), Lebert (18), and Schimkewitsch (33). These were followed in the twentieth century by Lendl (19), Nielsen (24), Kolosvary (17), Savory (32), and Caporiaccio (9). The studies culminated in the only complete description of the musculature of one species known to the authors—that of Brown (7), working with *Agelena naevia*.

<sup>1</sup>Manuscript received June 16, 1959.

Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, based largely on a thesis submitted by the first author in partial fulfillment of the requirements for the degree of Master of Arts. This project was supported financially by the National Research Council of Canada.

This paper was read at the 10th International Congress of Entomology in August, 1956, at Montreal, Que., Canada.

Valuable information on musculature was accumulated by the many students who concentrated on the separate systems in the araneid body. These will be mentioned in the discussions of the muscles of the various systems in *Latrodectus mactans*.

### Materials and Methods

One hundred adult female spiders were imported from Texas in August 1954. They were housed in pint jars covered with wire screening, and fed on house flies. Egg cocoons were collected, and spiderlings kept in cotton-stoppered vials and fed with *Drosophila* until large enough to be given house flies. Adult females averaged 11 mm in body length, while adult males were only 4 mm in length.

To study the morphology of the exoskeleton, adult spiders were cleared overnight in 10% potassium hydroxide. The cephalothoracic endosternite was observed by means of dissection of preserved material. Muscles were first studied in serial sections of immature females. When the difficulties of sectioning the heavily sclerotized exoskeleton of adult females had been overcome, further studies were carried out on the adults. Sections of male adults allowed comparison to be made with the female musculature.

The technique of sectioning adults was based on the general method of preparing paraffin sections, but with the following special features:

1. Spiders were killed by heating in distilled water over a boiling water bath. Heating was continued until the appendages were extended, but care was required that the abdominal contents did not expand enough to rupture the exoskeleton. (It was noted incidentally that in the specimens killed by heating, rather than by a simple immersion in the fixative, the pigment of the eyes was partially removed.)

2. In order to facilitate penetration of fluids into the body, the legs were cut off between the coxa and trochanter, or the trochanter and femur. The tips of the tarsi were removed from most legs which were to be sectioned. After 24 hours in Susa's fixative, and 12 hours in 80% ethyl alcohol, further steps were taken to ensure thorough permeation. With immature females and mature males, the abdomen was pricked on the lateral surfaces with a fine needle, while with adult females, thin slices were removed from each side of the abdomen with a razor blade.

3. A 12-hour immersion in the phenolic "B" component of Petrunkevitch's spider fixative seemed to render the internal structures less susceptible to hardening under later processes.

4. After additional washings in 80% ethyl alcohol for a total of 24 hours, dehydration was carried out in ethyl alcohol for another 24 hours, while clearing was accomplished in two baths of cedarwood oil for a total of 7 days. The oil was removed by two benzol baths in a total of 48 hours.

5. The specimens were infiltrated with melted Tissuemat at a temperature of 55° C for one bath of 8 to 12 hours, and another of 3 hours. After

the specimens were embedded in fresh Tissuemat, the hardened blocks were trimmed to expose one surface of the specimen, and soaked in water for at least 4 days. This latter procedure completed the process of softening hard structures sufficiently to allow easy sectioning.

The specimens were sectioned at thicknesses of 6 to 20 micra,  $10\mu$  being the average. Sections in the vertical, transverse, and horizontal planes were mounted on clean glass slides, using Mayer's albumen.

Staining by means of Delafield's haematoxylin, with eosin as the counterstain, gave the best general results. Mallory's triple stain was used to study the endoskeleton, and to intensify the striations of the muscle fibers. The sections were mounted in diaphane.

Drawings of sections were made with the aid of a Bioscope projector. All drawings are semidiagrammatic, and unless otherwise stated, are of the adult female black widow spider.

The description of the musculature has been supplemented with a brief account of the exoskeleton and endoskeleton, in order to clarify the nature and position of the points of attachment of the muscles. The nomenclature used for the muscles is a synthesis of functional and segmental systems. Each muscle has been named according to a function deduced from the points of attachment of the muscle. If there is a segmental arrangement of the muscles within a functional system, that fact has been indicated by appending the number of the segment to the name of the muscle, using Roman numerals.

Each muscle has also been assigned a number, by means of which it is labelled in the drawings. Here, too, the segmental nature of the muscles has been indicated by means of Roman numerals. A list of the muscles described, together with their numbers, points of origin and insertion, and homologous muscles, if any, described in the other species of spiders, will be found in Tables I-XVI. Unless otherwise stated, each muscle is paired.

### Morphology

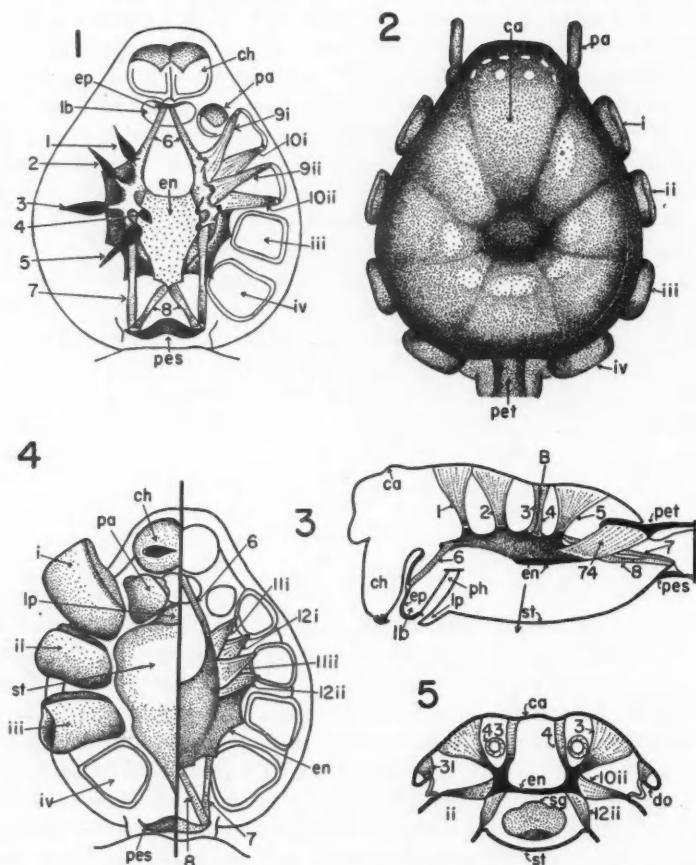
Although a detailed histological examination of the muscular tissue was not undertaken, it might be noted that in *Latrodectus mactans* there appears to be a continuous series of histological types of muscular and muscle-like tissue, from the striated muscle fibers typical of arthropods, through the fibers which differ from these only in the lack of striations, to the connective tissue tendons, which may be differentiated by the use of Mallory's stain.

There are two main types of muscles to be found in the body of a spider: those muscles with definite points of origin and insertion, one of these points usually being on the exoskeleton or the endoskeleton; and those muscles which form part of the walls of such structures as the venom gland and the heart. The muscles of the first type often insert by means of a connective tissue tendon, especially if the muscle is long and relatively slender.

## A. CEPHALOTHORAX

## 1. EXOSKELETON

The cephalothorax of an adult spider is developed from the prostomium, representing the true head, and six embryological segments as follows: the cheliceral segment, a segment bearing the pedipalps, and four segments bearing the legs. The tergites of the segments have fused to form the carapace (*ca*, Fig. 2), a heavily sclerotized plate which is arched over the cephalothorax, curving sharply down anteriorly to form the "facial region". The eight eyes are situated in two horizontal rows of four at the top of this curve, on an elevated plane which rises and broadens from a central depression in the carapace. This, with other depressions, marks the origins of some of the



FIGS. 1-5. Fig. 1. Dorsal view of cephalothorax, after removal of carapace. Fig. 2. Dorsal view of cephalothorax. Fig. 3. Sagittal view of cephalothorax and pedicel. Fig. 4. Ventral view of cephalothorax after removal of left half of sternum. Fig. 5. Cross-section of cephalothorax, as indicated at B, Fig. 3. For explanation of lettering on figures see page 870.

major cephalothoracic muscles. The lateral and posterior edges of the carapace are bent under to form a narrow shelf, the doublure (*do*, Fig. 5).

The ventral counterpart of the carapace is the sternum (*st*, Fig. 4), a smaller plate lying between the coxae of the legs. A small anterior lobe of the sternum is known as the lower lip (*lp*, Fig. 3), as it is situated immediately posteriorly to the buccal cavity.

The carapace and sternum are joined by a flexible portion of the exoskeleton known as the arthrodial membrane, since the bases of the appendages articulate with the body by means of this membrane.

There are six pairs of cephalothoracic appendages: the chelicerae, the palpi, and four pairs of walking legs.

The chelicerae migrate during their embryological development from a postoral position to their situation at the front of the cephalothorax, inserting beneath the overhanging anterior surface of the carapace (*ch*, Figs. 1, 3, 4).

The basal segments of the palpi (*pa*, Figs. 1, 4) lie immediately posteriorly and slightly laterally to the chelicerae. The arthrodial membrane between the palpal coxae is expanded ventrally, forming the labrum (*lb*, Figs. 1, 3). This structure lies posteriorly to the chelicerae, and anteriorly to the pharynx. Although not usually considered to be a true appendage, the labrum of the black widow spider was recently shown by Rempel (31) to develop from paired rudiments, each with a coelomic sac. A sclerotized plate on the anterior wall of the labrum, connecting the two palpal coxae, is called the epistome (*ep*, Figs. 1, 3). The coxae of the palpi are also expanded ventrally as the coxal processes or "maxillae" (*pa*, Fig. 4), which furnish the sides of the buccal cavity.

The coxae of the walking legs (*i*, *ii*, etc., Fig. 2) insert in two semicircles around the lateral edges of the sternum, and beneath the doublure of the carapace. Their articulations with the arthrodial membrane lie in an oblique plane, slanting ventrad and mesiad from the carapace. The articulation of the first leg is more nearly in the vertical plane than that of the fourth leg.

The fore-gut, which represents an ectodermal invagination, may be considered briefly here. The pharynx (*ph*, Fig. 3) is a cavity lying immediately posteriorly to the labrum and is expanded laterally behind the palpal coxae. It passes dorsad and posteriad to terminate in a constriction marking the beginning of the esophagus (*es*, Fig. 37). The latter is a narrow tube, almost circular in cross-section, running posteriorly through the cephalic ganglia to the sucking stomach (*ss*, Fig. 37). This organ lies above the main plate of the endosternite. In cross-section it has a form similar to that of a doubly concave lens which is thicker at the top than at the bottom (Fig. 38C).

## 2. ENDOSKELETON

The endoskeleton of the cephalothorax is the large endosternite (*en*, Figs. 1, 3, 4, 5), a flattened plate of mesodermal origin suspended in the center of the cephalothoracic cavity. It possesses two dorsal ridges which diverge from the tapered posterior tip of the plate, and extend anteriorly, forming the

anterior arms of the endosternite. These pass around the lateral edges of the brain. There are five dorsal arms arising from each dorsal ridge. The first three are directed obliquely dorsad and laterad, while the fourth, which arises mesially to the third, and the fifth are directed dorsad and mesiad. Laterally to each ridge the plate tapers out laterad and ventrad into irregular lateral wings.

### 3. MUSCULATURE

#### (a) *Endosternite* (Figs. 1, 3, 4, 5, and Table I)

Most of the muscles in the cephalothorax are attached to the endosternite. Eight paired muscles anchor the organ dorsally, anteriorly, and posteriorly. There is no muscular connection between the endosternite and the sternum, but the mesodermal plate is secured by other muscles which will be considered with the musculature of the structures upon which they exert their major action.

TABLE I  
Muscles of the cephalothoracic endosternite

| Muscle            | No. | Homologue* | Origin              | Insertion                      |
|-------------------|-----|------------|---------------------|--------------------------------|
| Suspensor i       | 1   | 39, 39A    | Carapace            | Dorsal arm i                   |
| Suspensor ii      | 2   | 49         | Carapace            | Dorsal arm ii                  |
| Suspensor iii     | 3   | 59         | Carapace            | Dorsal arm iii                 |
| Suspensor iv      | 4   | (Millot)   | Carapace            | Dorsal arm iv                  |
| Suspensor v       | 5   | 69         | Carapace            | Dorsal arm v                   |
| Protractor†       | 6   | 17         | Epistome            | Medial surface of anterior arm |
| Dorsal retractor  | 7   | 19         | Sternite of pedicel | Base of dorsal arm v           |
| Ventral retractor | 8   | 18         | Sternite of pedicel | Posterior tip of endosternite  |

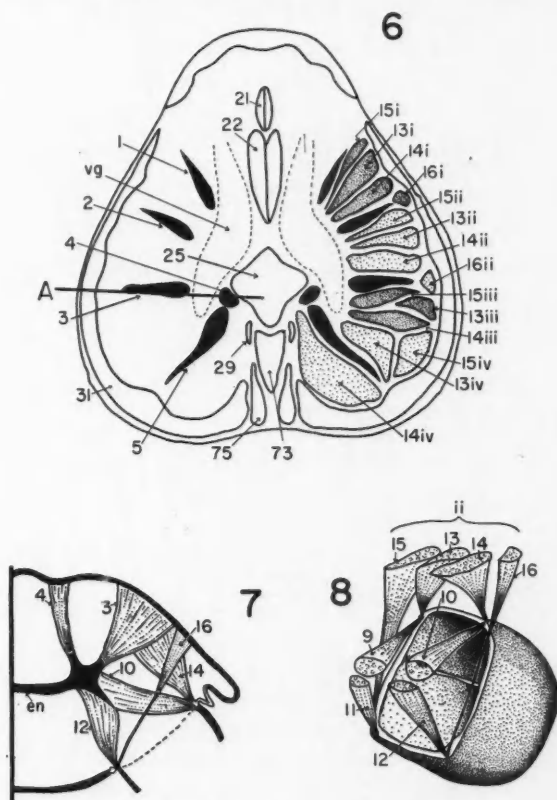
\*With the exception of suspensor iv, whose homologue was described by Millot (23) in *Lycosa*, all homologues shown were described by Brown (7) in *Agelena naevia*.

†This muscle also serves to compress the labral cavity.

#### (b) *Cephalothoracic Appendages*

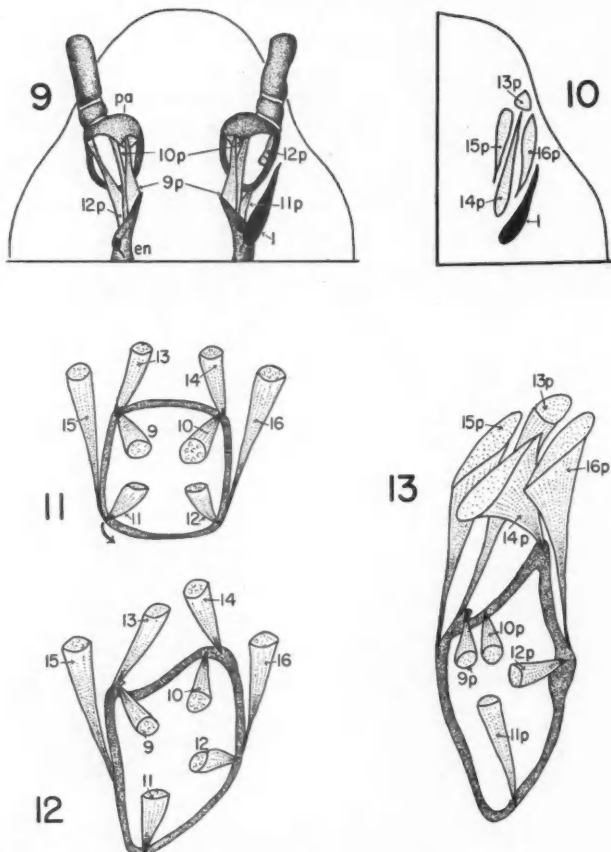
##### I. *Extrinsic Muscles*

(i) *Walking legs* (Figs. 6, 7, 8, and Table II).—The articulations of the coxae of the walking legs with the cephalothorax are almost rectangular in form. The muscles insert at the four corners of the bases of the coxae by means of tendons. Each corner of the coxa receives a muscle from the endosternite and one from the carapace (Figs. 7, 8). The posterior ventral corners of the coxae of the third and fourth legs lack a muscle from the carapace, but with this exception, the extrinsic muscles of the walking legs are uniform. The muscles coming to the coxae from the carapace lie in groups between the suspensors of the endosternite (Fig. 6). The muscles of each coxa are in a separate group, with the exception of the anterior levator (13 iv) and the anterior rotator (15 iv) of the fourth leg, which lie with the muscles of the third leg between the third and fifth suspensors of the endosternite.



FIGS. 6-8. Fig. 6. Dorsal plan of carapace, showing origins of muscles. Fig. 7. Cross-section of cephalothorax, as indicated at A, Fig. 6. Fig. 8. Second right leg, viewed from a posteromedial angle.

(ii) *Palpi* (Figs. 9-13 and Table III).—The extrinsic musculature of each palp consists of eight muscles which are homologous with those of the coxae of the walking legs. However, since the palpal coxae have undergone a ventral expansion forming the coxal processes, the points of insertion of some of the muscles have become changed. This signifies an altered function for these muscles. In addition, the articulation of the palp with the cephalothorax lies in a vertical plane parallel with the anterior surface of the cephalothorax so that the anterior surface of the coxa may now be called medial, and the posterior surface, lateral. Figures 11, 12, and 13 show the steps of the process which we suggest have brought about the following modifications: the ventral median corner of the articulation grows ventrad, due to the expansion of the ventral portion of the median surface of the articulation. This growth is so extensive that the muscle corresponding to the anterior depressor (11) of the leg is carried to a ventrolateral position. Meanwhile



FIGS. 9-13. Fig. 9. Dorsal view of anterior portion of cephalothorax after removal of carapace. Fig. 10. Dorsal plan of right anterior quadrant of carapace, showing origins of muscles. Figs. 11, 12, 13. Steps in proposed method of modification of leg coxa to form palpal coxa. Fig. 11 represents leg coxa, Fig. 13, palpal coxa.

the dorsal median corner bends anteriorly through almost 180 degrees until it is directed laterad. This carries the muscle homologous with the protractor (9) of the leg and that homologous with the anterior levator (13) in an anterior and lateral direction. Finally, the dorsal-lateral corner of the articulation grows dorsad and curves posterad to form a partial dome over the whole articular area (Fig. 9). The result of these changes is that all muscles but one (11), which is carried ventrad, insert in the dorsal region of the coxa. Therefore, the functions of the muscles are no longer the same as their homologues' functions. For this reason, and because of the plane of the articulation, the names of the muscles have been changed, while the numbers assigned to them remain the same to indicate the proposed homologies.

TABLE II  
Extrinsic muscles of the walking legs

| Muscle              | No. | Homologue* | Origin   | Insertion                     |
|---------------------|-----|------------|--|-------------------------------|
| Protractor          | 9   | Pro.       | Dorsal surface of lateral wings of endosternite  | Anterodorsal corner of coxa   |
| Retractor           | 10  | Re.        | Dorsal surface of lateral wings of endosternite  | Posterodorsal corner of coxa  |
| Anterior depressor  | 11  | D.a.       | Ventral surface of lateral wings of endosternite | Anteroventral corner of coxa  |
| Posterior depressor | 12  | D.p.       | Ventral surface of lateral wings of endosternite | Posteroventral corner of coxa |
| Anterior levator    | 13  | L.a.       | Carapace   | Anterodorsal corner of coxa   |
| Posterior levator   | 14  | L.p.       | Carapace   | Posterodorsal corner of coxa  |
| Anterior rotator    | 15  | R.a.       | Carapace   | Anteroventral corner of coxa  |
| Posterior rotator†  | 16  | R.p.       | Carapace   | Posteroventral corner of coxa |

\*All homologues shown were described by Steinbach (36) in *Filistata*.

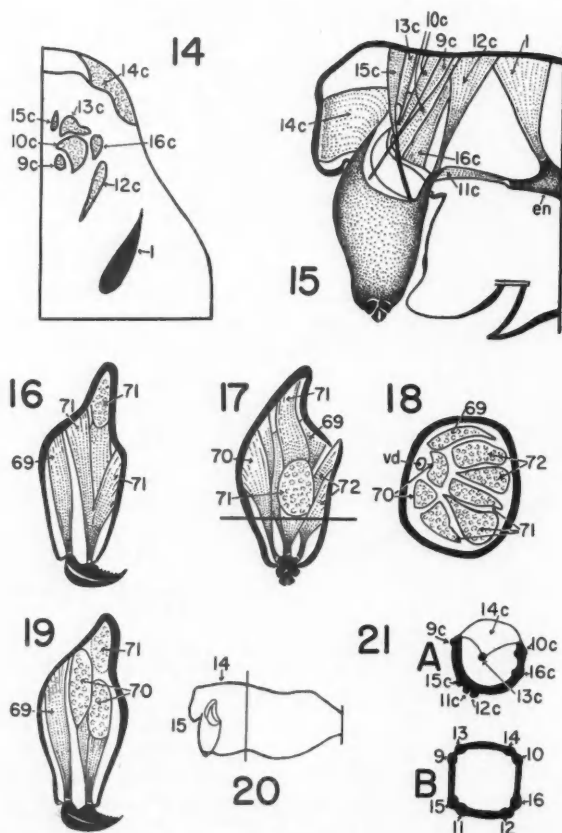
†The posterior rotator, which is a small muscle inserting by means of a long tendon, is absent in legs iii and iv; otherwise the muscles are present in all four pairs of legs, and may be distinguished by the use of the appropriate Roman numeral.

TABLE III  
Extrinsic muscles of the palpi

| Muscle           | No. | Homologue* | Origin  | Insertion                                   |
|------------------|-----|------------|---|---|
| Median adductor  | 9p  | Pro.       | Lateral surface of anterior arm of endosternite | Dorsomedian corner of coxa                  |
| Lateral adductor | 10p | Re.        | Ventral surface of anterior arm of endosternite | Dorsal edge of coxa, laterally to 9p        |
| Depressor        | 11p | D.a.       | Ventral surface of anterior arm of endosternite | Ventrally on lateral edge of coxa           |
| Abductor         | 12p | D.p.       | Lateral surface of anterior arm of endosternite | Lateral edge of coxa, dorsally to 11p       |
| Anterior rotator | 13p | L.a.       | Carapace  | Dorsomedian corner of coxa, with 9p         |
| Lateral levator  | 14p | L.p.       | Carapace  | Dorsal edge of coxa, laterally to 10p       |
| Median rotator   | 15p | R.a.       | Carapace  | Dorsomedian corner of coxa, medially to 13p |
| Lateral rotator  | 16p | R.p.       | Carapace  | Lateral edge of coxa, with 12p              |

\*All homologues shown were described by Steinbach (36) in *Filistata*.

(iii) *Chelicerae* (Figs. 14, 15, 21, and Table IV).—Eight muscles operate the basal segment of the chelicera. Here, however, only one of the muscles originates on the endosternite, the remaining seven all arising on the carapace. Following the homologies suggested by Steinbach (36) the numbers used will be the same as those of the corresponding muscles of the legs, while the names have been changed in accordance with the different plane of articulation and the modified points of attachment of some of the muscles.



FIGS. 14-21. Fig. 14. Dorsal plan of right anterior quadrant of carapace, as indicated in Fig. 20, and showing origins of muscles. Fig. 15. Sagittal view of anterior portion of cephalothorax, as indicated in Fig. 20. Fig. 16. Anterior view of right chelicera, after removal of medial wall. Fig. 17. Medial view of right chelicera after removal of anterior wall and muscle 70. Fig. 18. Cross-section of right chelicera, as indicated in Fig. 17. Fig. 19. Anterior view of right chelicera, after removal of anterior wall. Fig. 20. Sagittal view of cephalothorax. Fig. 21. A, proximal rim of basal segment of chelicera; B, corresponding view of proximal rim of coxa of leg.

TABLE IV  
Extrinsic muscles of the chelicerae

| Muscle                     | No. | Homologue* | Origin  | Insertion   |
|----------------------------|-----|------------|---|---|
| Levator                    | 9c  | Le.        | Carapace  | Anteromedian corner of basal segment                                |
| Anterior abductor          | 10c | Abd. a.    | Carapace  | Anterior half of lateral edge of basal segment                      |
| Posterior median adductor  | 11c | Add. p.m.  | Median surface of anterior arm of endosternite            | Laterally of posteromedian corner of basal segment                  |
| Posterior lateral adductor | 12c | Add. p.l.  | Carapace  | On short projection, with 11c                                       |
| Anterior median adductor   | 13c | Add. a.m.  | Carapace  | Anterior wall of basal segment (dorsal tip)                         |
| Anterior lateral adductor  | 14c | Add. a.l.  | Anteriorly and laterally on curved "face" of the carapace | Dorsal portions of anterior and anterolateral wall of basal segment |
| Depressor†                 | 15c | De.        | Carapace  | Fused posteromedian corners of basal segment                        |
| Posterior abductor         | 16c | Abd. p.    | Carapace  | Posterolateral portion of edge of basal segment                     |

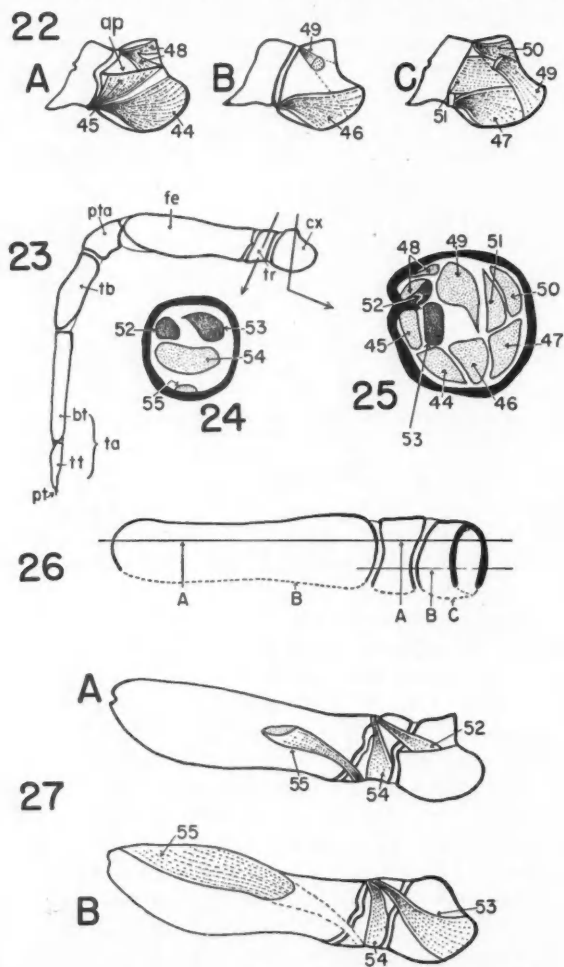
\*All homologues shown were described by Steinbach (36) in *Filistata*.

†The two members of the pair insert by means of a common tendon.

## II. Intrinsic Muscles and Articulations

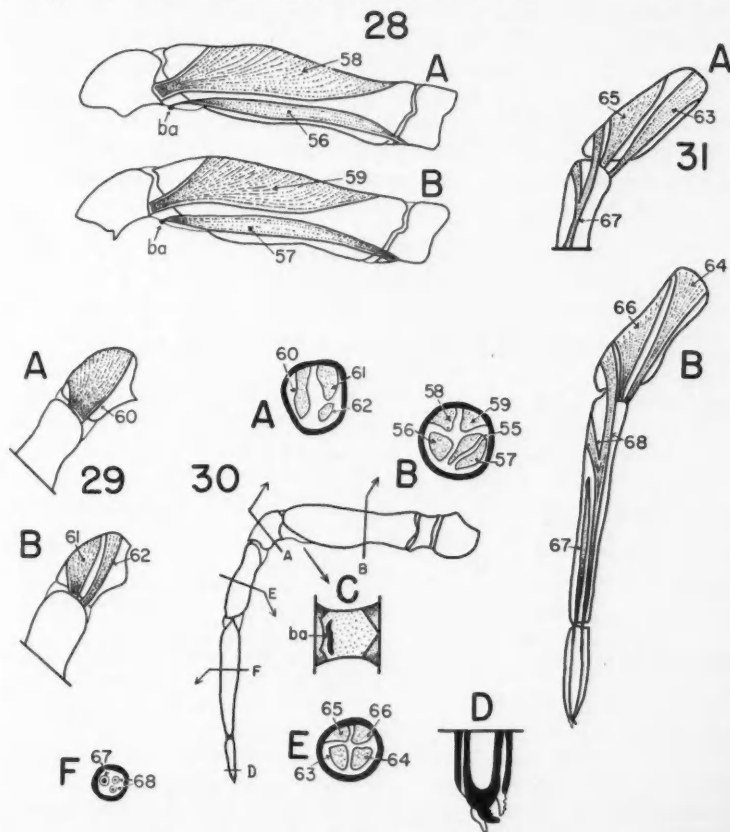
(i) *Walking legs* (Figs. 22-31 and Table V).—Each pair of walking legs differs slightly in length and in the angle formed with the longitudinal axis of the body, but the segmentation and intrinsic musculature of the legs is uniform. There are seven segments (Fig. 23): coxa (*cx*), trochanter (*tr*), femur (*fe*), patella (*pta*), tibia (*tb*), tarsus (*ta*), and pretarsus (*pt*). The tarsus is subdivided into two tarsomeres, the basitarsus (*bt*) and the telotarsus (*tt*), the two "segments" known also as metatarsus and tarsus. These two divisions are not considered to be true segments, however, as no muscular connection occurs between them. The types of articulation between the segments are varied to allow several kinds of movement. There seems to be no definite point of articulation between the coxa and the cephalothorax, for although slight protuberances may be seen on the lateral edges of the sternum, at points corresponding to the posterior ventral corners of the coxae, there are no corresponding structures on the coxae. That movement is possible in all directions may be concluded from the strong extrinsic musculature of the coxae, the muscles inserting at the four corners of the articulation. The trochanter articulates with the coxa by means of a condylic joint on the anterior surface of the segments. The anterior apodeme (*ap*, Fig. 22A) of the coxa is extended distally to fit into a corresponding cavity on the anterior surface of the proximal rim of the trochanter. All the muscles operating the trochanter arise in the proximal portion of the coxa and insert on the proximal rim of the trochanter (Figs. 22, 25). There are four depressors, three levators, and a reductor. Therefore, while the main movement is

in the vertical plane, the anterior articulation does allow a lateral movement in the posterior direction. The femur moves on the trochanter by means of a dicondylic articulation on the lateral walls of the segments, in the dorsal half. The vertical movement of the femur is brought about by three levators and one strong depressor. Two of these muscles arise in the coxa, and two



FIGS. 22-27. Fig. 22. A, coxa and trochanter of leg, viewed posteriorly at level A, as indicated in Fig. 26; B, the same, viewed at level of B, Fig. 26; C, the same, viewed at level C, Fig. 26. Fig. 23. Posterior view of walking leg. Fig. 24. Cross-section of trochanter, as indicated in Fig. 23. Fig. 25. Cross-section of coxa, as indicated in Fig. 23. Here and in Fig. 24, shaded muscles act on the femur, unshaded muscles, on the trochanter. Fig. 26. Dorsal plan of coxa, trochanter, and femur of walking leg. Fig. 27. A, coxa, trochanter, and femur, viewed posteriorly at level A, as indicated in Fig. 26; B, the same, viewed at level B, Fig. 26.

in the trochanter (Figs. 24, 25, 27). The joint between the femur and the patella is also dicondylic, and is in the dorsal portion of the segments. There is a small arcuate sclerotized bar in the arthrodial membrane between the femur and patella (*ba*, Fig. 30C). Of the muscles acting on the patella, two arise in the trochanter, inserting on the arcuate bar, and two in the femur; all four are depressors (Figs. 28, 30B). This latter fact, in combination with the presence of the dorsal dicondylic hinge between the patella and the femur, suggests that this joint is the main point of flexure in the leg of the black widow. The tibia moves on the patella by means of a unique dorsal articulation consisting of an oblique hinge which allows movement in the horizontal plane. However, the tightness of the arthrodial membrane here



FIGS. 28-31. Fig. 28. A, posterior view of anterior half of trochanter, femur, and patella; B, posterior view of posterior half of the same. Fig. 29. A, posterior view of anterior half of patella and proximal half of tibia; B, posterior view of posterior half of the same. Fig. 30. Posterior view of walking leg. A, B, E, F, cross-sections of leg; C, ventral view of leg, between femur and patella; D, enlarged view of pretarsus of leg, as indicated in Fig. 30. Fig. 31. A, posterior view of anterior half of tibia and tarsus; B, posterior view of posterior half of the same.

prevents movement of any great magnitude. Corresponding to the type of articulation, the three muscles which originate in the patella and insert on the lateral edges of the tibia produce movement of the latter segment in the horizontal plane (Figs. 29, 30A). The basitarsus articulates with the tibia by means of a dorsal dicondylic joint similar to that between the femur and the patella, just as the four muscles which arise in the tibia and insert ventrally on the basitarsus are comparable to the four depressors of the patella (Figs. 30E, 31). The extent of the arthrodistal membrane proximal to the basitarsus is less, however, so the magnitude of the flexure is also smaller. Although, as previously mentioned, no muscles connect the basitarsus to the telotarsus, there does appear to be a dorsal dicondylic joint between the two tarsomeres. The articulation of the pretarsus with the telotarsus was not clearly observed due to the small size of the distal segment, but the musculature would suggest a joint, probably dicondylic, allowing movement in a vertical plane. Both the flexor and the extensor arise by means of two heads, one on the dorsal surface of the distal area of the tibia, and one on the proximal area of the dorsal surface of the basitarsus (Figs. 30D, 30F, 31). Because both muscles arise partially in the tibia, they could possibly act to extend the tarsus as a whole, a suggestion made by Dillon (10).

TABLE V  
Intrinsic muscles of the walking legs

| Muscle                     | No. | Homologue* | Origin   | Insertion                                    |
|----------------------------|-----|------------|--|--|
| <b>Trochanter</b>          |     |            |  |  |
| Ventral anterior depressor | 44  | 1, 2       | Ventral surface of coxa at the proximal anteroventral corner                         | Proximal anteroventral rim of trochanter     |
| Dorsal anterior depressor  | 45  | 3, 5       | Ventral surface of anterior apodeme of coxa  | Proximal anteroventral rim of trochanter     |
| Median depressor           | 46  | 7          | Proximal ventral surface of coxa   | Proximal ventral rim of trochanter           |
| Posterior depressor        | 47  | 8          | Posteroventral corner of coxa  | Posteroventral corner of trochanter          |
| Anterior levator           | 48  | 4          | Proximal part of dorsal surface of anterior apodeme and antero-dorsal corner of coxa | Anterodorsal corner of trochanter            |
| Median levator             | 49  | 6          | Posteroventral corner of coxa  | Proximal dorsal rim of trochanter            |
| Posterior levator          | 50  | 10         | Posterodorsal corner of coxa   | Posterodorsal corner of trochanter           |
| Reductor                   | 51  | R.tr. ?    | Proximal rim of posterior wall of coxa   | Proximal rim of posterior wall of trochanter |
| <b>Femur</b>               |     |            |  |  |
| Anterior dorsal levator    | 52  | 6 ?        | Distal part of dorsal surface of anterior apodeme of coxa                            | Anterodorsal corner of femur                 |
| Posterior dorsal levator   | 53  | Fl.f.l.    | Medially on proximal part of ventral surface of coxa                                 | Medially to 52                               |
| Ventral levator            | 54  | 7          | Proximal part of ventral wall of trochanter  | Dorsal wall of proximal rim of femur         |

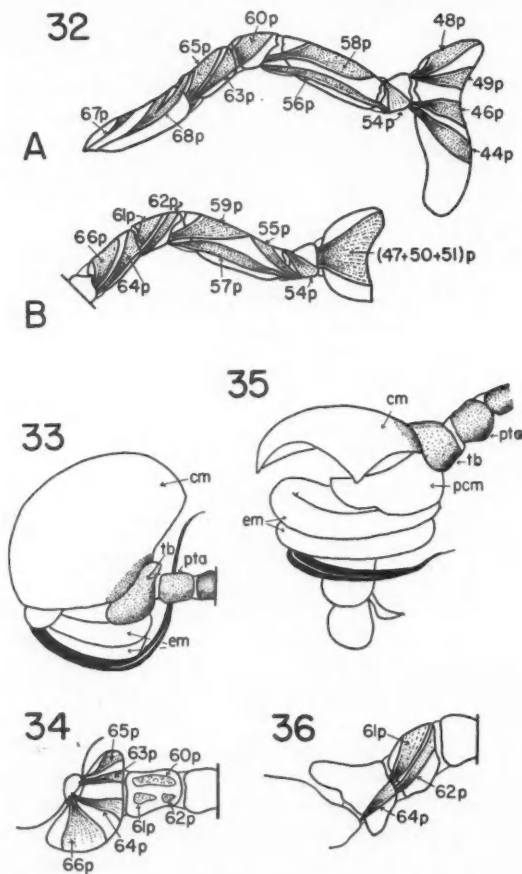
TABLE V (Concluded)  
Intrinsic muscles of the walking legs

| Muscle                      | No. | Homologue* | Origin   | Insertion  |
|-----------------------------|-----|------------|--|--|
| Depressor                   | 55  | 8          | Distal ventral lip of trochanter   | Posterior half of dorsal wall of femur                                       |
| Patella                     |     |            |  |  |
| Anterior ventral depressor  | 56  | 11         | Anteroventral corner of trochanter   | Anterior end of arcuate bar in arthrodial membrane between femur and patella |
| Posterior ventral depressor | 57  | 12         | Posteroventral corner of trochanter  | Posterior end of arcuate bar   |
| Anterior dorsal depressor   | 58  | 9          | Anterior part of dorsal wall of femur  | Anteroventral corner of patella  |
| Posterior dorsal depressor  | 59  | 10         | Posterior part of dorsal wall of femur, medially to 55   | Posteroventral corner of patella   |
| Tibia                       |     |            |  |  |
| Productor                   | 60  | 14         | Anterior half of dorsal wall of patella  | Ventral half of anterior proximal rim of tibia                               |
| Dorsal reductor             | 61  | 15         | Posterior half of dorsal wall of patella   | Posterior proximal rim of tibia  |
| Ventral reductor            | 62  | 16         | Proximally to 61   | Ventrally to 61  |
| Basitarsus                  |     |            |  |  |
| Anterior ventral depressor  | 63  | 19         | Anterior proximal rim of tibia   | Anteroventral corner of basitarsus   |
| Posterior ventral depressor | 64  | 20         | Posterior proximal rim of tibia  | Posteroventral corner of basitarsus  |
| Anterior dorsal depressor   | 65  | 17         | Anterior half of dorsal wall of tibia  | Slightly dorsally to 63  |
| Posterior dorsal depressor  | 66  | 18         | Posterior half of dorsal wall of tibia   | Slightly dorsally to 64  |
| Pretarsus                   |     |            |  |  |
| Extensor                    | 67  | 21         | Anterior part of dorsal distal surface of tibia and anterior part of dorsal proximal surface of basitarsus   | Dorsally on pretarsus  |
| Flexor                      | 68  | 22         | Posterior part of dorsal distal surface of tibia and posterior part of dorsal proximal surface of basitarsus | Ventrally on pretarsus   |

\*The homologues of the muscles of the trochanter were described by Dillon (10) in *Miranda*, with the exception of the reductor, and the homologues of the other muscles were described by Snodgrass (35) in *Eurypelma*, with the exception of posterior dorsal levator of the femur which, like the reductor of the trochanter, was described in *Agelena naevia* by Brown (7), who quoted Petrunkevitch.

(ii) *Palpi* (Figs. 32-36 and Table VI).—The female palp consists of the same seven segments as the leg: coxa, trochanter, femur, patella, tibia, tarsus, and pretarsus. However, the tarsus is undivided. The coxa is modified as described previously, and the articulations between the segments are identical with those of the leg, except that the arcuate bar is not present in the arthrodial membrane between the femur and the patella. Since the palpi are directed anteriorly, the surface corresponding to the anterior surface of the legs has become median, and that corresponding to the posterior surface of the legs, lateral. Therefore the names of the muscles have been changed,

while the numbers assigned to them indicate their homologies with the muscles of the leg. The modification of the palpal coxa has been accompanied by changes in the muscles which lie within its cavity, and which act on the trochanter. The two muscles which pass from the coxa to the femur in the leg are not present in the palp. The remaining muscles of the female palp, however, are similar to their homologues in the leg, with the two following exceptions: the ventral depressors of the patella insert directly on the proximal rim of the patella, as the arcuate bar is not present, and the extensor of the pretarsus lacks a branch to the tibia. The male palp is made up of the same seven segments as the female palp, but the three distal segments have become



FIGS. 32-36. Fig. 32. A, lateral view of medial half of left palp of female; B, lateral view of lateral half of the same. Fig. 33. Dorsal view of palp of male. Fig. 34. Enlarged view of portions shaded in Fig. 33, after removal of dorsal walls to show musculature. Fig. 35. Lateral view of palp of male. Fig. 36. Enlarged view of portions shaded in Fig. 35, after removal of lateral walls, to show musculature.

modified to form a copulatory organ (Figs. 33, 35). The tibia of the male palp has become a short segment, expanded laterally and slightly dorsally and ventrally to curve around the insertion of the tarsus. The latter is a flattened plate known as the cymbium (*cm*, Fig. 33), with a ventral groove to receive the greatly enlarged pretarsus, known in the male as the embolus (*em*, Fig. 33). Between the cymbium and the embolus is a small pseudosegment, the paracymbium (*pcm*, Fig. 35). With the exception of the muscles operating the distal two segments, the musculature of the male palp is identical with that of the female palp. The expansion of the tibia has carried the origins of the two dorsal depressors of the tarsus to the lateral surfaces of the segment. These two muscles still insert with the ventral depressors on the ventral proximal rim of the tarsus, or cymbium. No muscles are seen to act on the pretarsus, or embolus, of the male palp in *Latrodectus mactans*.

TABLE VI  
Intrinsic muscles of the palpi\*

| Muscle                    | No.                 | Origin  | Insertion                                       |
|---------------------------|---------------------|---|---|
| Trochanter                |                     |   |   |
| Ventral depressor         | 44 <i>p</i>         | Posterior surface of coxa   | Ventromedial corner of trochanter               |
| Adductor                  | 45 <i>p</i>         | Medial surface of coxa, near epistome                                 | Ventromedial corner of trochanter               |
| Dorsal depressor          | 46 <i>p</i>         | Proximal lateral rim of coxa  | Ventrolateral corner of trochanter              |
| Dorsal levator            | 48 <i>p</i>         | Dorsal wall of coxa, near median line                                 | Dorsomedial corner of trochanter                |
| Ventral levator           | 49 <i>p</i>         | Dorsally to 46 <i>p</i>   | Dorsolateral corner of trochanter               |
| Abductor                  | (47+50+51) <i>p</i> | Proximal lateral rim of coxa, distally to 46 <i>p</i> and 49 <i>p</i> | Lateral rim of trochanter                       |
| Femur                     |                     |   |   |
| Levator                   | 54 <i>p</i>         |   | As in walking leg†                              |
| Depressor                 | 55 <i>p</i>         |   | As in walking leg†                              |
| Patella                   |                     |   |   |
| Median ventral depressor  | 56 <i>p</i>         | As in walking leg   | Ventromedial corner of proximal rim of patella  |
| Lateral ventral depressor | 57 <i>p</i>         | As in walking leg   | Ventrolateral corner of proximal rim of patella |
| Median dorsal depressor   | 58 <i>p</i>         |   | As in walking leg                               |
| Lateral dorsal depressor  | 59 <i>p</i>         |   | As in walking leg                               |
| Tibia                     |                     |   |   |
| Adductor                  | 60 <i>p</i>         |   | As in walking leg                               |
| Dorsal abductor           | 61 <i>p</i>         |   | As in walking leg                               |
| Ventral abductor          | 62 <i>p</i>         |   | As in walking leg                               |
| Female tarsus             |                     |   |   |
| Median ventral depressor  | 63 <i>p</i>         |   | As in walking leg                               |
| Lateral ventral depressor | 64 <i>p</i>         |   | As in walking leg                               |
| Median dorsal depressor   | 65 <i>p</i>         |   | As in walking leg                               |
| Lateral dorsal depressor  | 66 <i>p</i>         |   |   |

TABLE VI (Concluded)  
Intrinsic muscles of the palpi\*

| Muscle                    | No. | Origin  | Insertion         |
|---------------------------|-----|---|-------------------|
| Male tarsus               |     |   |                   |
| Median ventral depressor  | 63p | As in walking leg   |                   |
| Lateral ventral depressor | 64p | As in walking leg   |                   |
| Median depressor          | 65p | Medial surface of tibia   | As in walking leg |
| Lateral depressor         | 66p | Lateral surface of tibia  | As in walking leg |
| Female pretarsus          |     |   |                   |
| Extensor                  | 67p | Medial part of dorsal surface of tarsus   | As in walking leg |
| Flexor                    | 68p | Two origins: distal medial part of dorsal surface of tibia and proximal medial part of dorsal surface of tarsus | As in walking leg |
| Male pretarsus            |     |   |                   |
| No muscles                |     |   |                   |

\*Because no detailed description of the intrinsic muscles of the palpi was found in the literature, no homologues are given here.

†It should be remembered that the palpi are directed in the direction of the longitudinal axis of the body, while the legs are more closely parallel to the horizontal axis. Therefore, the terms "anterior" and "posterior", as used in reference to the legs, become "medial" and "lateral", respectively, when applied to the palpi.

(iii) *Chelicerae* (Figs. 16-19 and Table VII).—The chelicerae have two segments, a ventrally directed basal segment, and a small, medially directed claw which articulates with the basal segment by means of a strong dicondylic joint, allowing movement in the vertical plane. This movement is brought about by three flexors and one extensor. The muscles arise on the proximal portions of the walls of the basal segment, usually with multiple origins, and insert by means of tendons on the proximal rim of the claw.

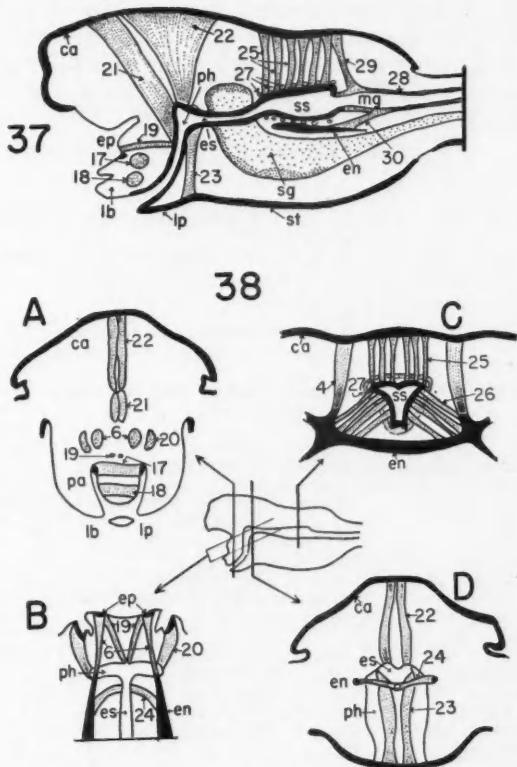
TABLE VII  
Intrinsic muscles of the chelicerae

| Muscle           | No. | Holomogue*               | Origin   | Insertion                                 |
|------------------|-----|--------------------------|--|---|
| Cheliceral claw  |     |                          |  |   |
| Extensor         | 69  | $m_5$                    | Dorsal area of lateral wall of basal segment   | Lateral proximal rim of claw              |
| Anterior flexor  | 70  | $m_4$<br>(three muscles) | Two origins: one beside the other, on the anterior wall of the basal segment                 | Anteromedial area of proximal rim of claw |
| Median flexor    | 71  |                          | Three origins: dorsal areas of anterior wall, medial wall, and lateral wall of basal segment | Medial proximal rim of claw               |
| Posterior flexor | 72  |                          | Two origins: one beside the other, on the posterior wall of the basal segment                | Posteriorly to 71                         |

\*The homologues shown were described by Schimkewitsch (33) in *Epeira*.

*(c) Digestive System (Figs. 37, 38, and Table VIII)*

There are 14 muscles contributing to the function of the digestive mechanism in the cephalothorax, 9 of which are paired.



FIGS. 37-38. Fig. 37. Sagittal section of cephalothorax. Fig. 38. A, B, C, D, sections of cephalothorax as indicated.

*(d) Carapace (Fig. 5 and Table IX)*

Passing dorsad from the doublure of the carapace to the dorsal surface of the carapace is a muscle made up of fibers which border the carapace from the level of the posterior eyes to that of the anterior projections of the lorum of the pedicel.

*(e) Coxal Gland (Figs. 39, 40, and Table IX)*

Typical of araneomorphic spiders, the coxal gland of the black widow consists of a muscular sacculus (*sa*) and a sclerotized labyrinth (*la*). The sacculus lies immediately ventrally to the anterior caecum of the mid-gut, at the point of origin of the blind caecum passing into the coxa of the first leg. The sacculus leads distally into the labyrinth, a short passage opening at the posteroventral corner of the articulation of coxa I.

The five dilator muscles of the sacculus are very weak fibers apparently continuous with its muscular wall. There are five muscles serving to dilate the opening of the labyrinth at the base of coxa I, the median dilator (40) being the strongest.

TABLE VIII  
Muscles of the digestive system

| Muscle                                  | No. | Homologue* | Origin   | Insertion  |
|---|-----|------------|--|--|
| Labrum                                  |     |            |  |  |
| Dorsal compressor†<br>(unpaired)        | 17  | 2          | Passes from one side of labrum to the other    |  |
| Ventral compressor<br>(unpaired)        | 18  | 1          | Similar to 17, but ventrally to it             |  |
| Pharynx                                 |     |            |  |  |
| Median dilator‡                         | 19  | 3          | Epistome, between protractors of endosternite  | Median channel in anterior wall of pharynx                             |
| Lateral dilator                         | 20  | 4          | Anterior wall of palpal coxa                   | Ventrolaterally on anterior wall of pharynx                            |
| Anterior dilator                        | 21  | 5          | Carapace                                       | Dorsally to 19   |
| Posterior dilator                       | 22  | 6          | Posteriorly to 21                              | Medial area of dorsal surface of pharynx                               |
| Ventral dilator                         | 23  | 7          | Sternum, posteriorly to lower lip              | Dorsal area of posterior wall of pharynx                               |
| Esophagus                               |     |            |  |  |
| Lateral dilator                         | 24  | 8          | Medial surface of anterior arm of endosternite | Anterior part of lateral wall of esophagus                             |
| Sucking stomach                         |     |            |  |  |
| Dorsal dilator<br>(apparently unpaired) | 25  | 9d         | Central depression of carapace                 | Dorsal surface of sucking stomach                                      |
| Lateral dilator                         | 26  | 9i         | Dorsal surface of endosternite                 | Lateral surface of sucking stomach                                     |
| Constrictor<br>(unpaired)               | 27  | 10         | Separate fibers encircling the sucking stomach |  |
| Mid-gut                                 |     |            |  |  |
| Constrictor<br>(unpaired)               | 28  | (Milot)    | Longitudinal fibers in mid-gut wall            |  |
| Dorsal dilator                          | 29  | —          | Carapace                                       | Continuous with fibers in dorsal wall of mid-gut, posteriorly to caeca |
| Ventral dilator                         | 30  | —          | Dorsal surface of endosternite                 | Similar to 29, but on ventral wall                                     |

\*With the exception of the constrictor of the mid-gut, which was described by Milot (23), all homologues shown were described by Brown (7) in *Agelena naevia*.

†This muscle is attached to the lateral portions of the epistome, and therefore to the medial surface of the palpal coxae, so it could also act to adduct the palpal coxal processes, or "maxillae".

‡This muscle, whose two members insert together, also serves to compress the labral cavity.

(f) *Venom Gland* (Figs. 5, 6, 42, and Table IX)

The venom gland of *Latrodectus mactans* is an elongate sac lying between dorsal arms, I, II, and III laterally, and the posterior dilator of the pharynx, the dorsal dilator of the sucking stomach, and dorsal arms IV and V of the

endosternite, medially (vg, Figs. 6, 42). The gland leads anteriorly to the venom duct, which passes ventrad along the anterior surface of the basal segment of the chelicera, into the claw, and opens on the convex surface of the claw, near the distal extremity. The gland has a heavy muscular wall consisting of striated fibers running longitudinally along the body of the gland. The fibers pass from the posterior tip of the gland to the constriction which marks the beginning of the venom duct. The twisting of the gland to fit into the space between the muscles and the dorsal arms of the endosternite gives the fibers the appearance of being arranged spirally.

TABLE IX  
Muscles of the carapace, coxal gland, and venom gland

| Muscle                     | No. | Homologue* | Origin  | Insertion                                    |
|----------------------------|-----|------------|---|--|
| Carapace<br>Compressor     | 31  | 20         | Lateral edges of dorsal surface of carapace     | Doublure of carapace                         |
| Sacculus of coxal gland    |     |            |   |  |
| Constrictor                | 32  | (Millot)   | Fibers running longitudinally in wall           |  |
| Anterior dilator           | 33  | —          | Dorsal arm i of endosternite                    | Anterior surface of sacculus                 |
| Posterior dilator          | 34  | —          | Dorsal arm iii of endosternite                  | Posterior surface of sacculus                |
| Lateral dilator <i>a</i>   | 35  | —          | Proximal dorsal rim of coxa i                   | Lateral surface of sacculus                  |
| Lateral dilator <i>b</i>   | 36  | —          | Posteriorly to 35                               | Posteriorly to 35                            |
| Lateral dilator <i>c</i>   | 37  | —          | Posteriorly to 36                               | Posteriorly to 36                            |
| Labyrinth of coxal gland   |     |            |   |  |
| Anterior dilator           | 38  | —          | Proximal anterior rim of coxa i                 | Anterior surface of distal end of labyrinth  |
| Lateral dilator            | 39  | —          | Proximal dorsal rim of coxa i                   | Lateral surface of distal end of labyrinth   |
| Median dilator             | 40  | —          | Dorsal arm i of endosternite                    | Dorsally to 38                               |
| Posterior dilator          | 41  | —          | Anteroventral corner of proximal rim of coxa ii | Posterior surface of distal end of labyrinth |
| Dorsal dilator             | 42  | —          | Dorsal arm ii of endosternite                   | Median surface of distal end of labyrinth    |
| Venom gland<br>Constrictor | 43  | (Kessler)  | Longitudinal striated fibers in wall            |  |

\*The homologue of the compressor of the carapace was described by Brown (7) in *Agelena naevia*, that of the constrictor of the sacculus of the coxal gland by Millot (23), and that of the constrictor of the venom gland by Kessler (16) in *Lycosa*.

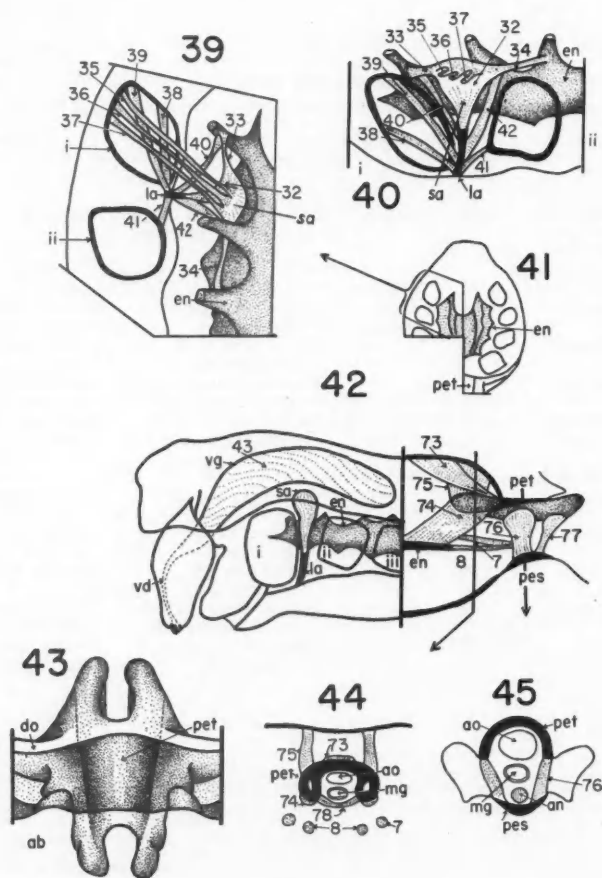
## B. PEDICEL

### 1. EXOSKELETON

(Figs. 42, 43)

Representing the seventh segment of the arachnid body, the pedicel is greatly constricted in comparison with the rest of the body, but still contains portions of the aorta (*ao*), the mid-gut (*mg*), and the abdominal nerve trunk

(*an*) seen in Fig. 45. The tergite or lorum of the pedicel (*pet*, Figs. 43, 44, 45) is curved over the segment from side to side, and bears terminal arms projecting anterad into the cephalothorax, and posterad into the abdomen. The lorum, therefore, assumes the shape of the letter H when viewed dorsally. The arthroal membrane, which connects the tergite to the small crescent-shaped sternite (*pes*, Figs. 42, 45), is carried with the terminal arms into the cephalothoracic and abdominal cavities, and forms part of the club-like projections of the pedicel.



FIGS. 39-45. Fig. 39. Dorsal view of cephalothorax after removal of carapace, as indicated in Fig. 41. Fig. 40. Lateral view of same portion of cephalothorax. Fig. 41. Dorsal view of cephalothorax, after removal of carapace. Fig. 42. Lateral view of cephalothorax, after removal of portion indicated in Fig. 41. Fig. 43. Dorsal view of pedicel. Figs. 44, 45. Cross-sections of pedicel, as indicated in Fig. 42.

## 2. MUSCULATURE

(Figs. 6, 42-45, and TABLE X)

There are three muscles, two of them paired, lying in the cephalothorax and serving to alter the angle between the pedicel and the cephalothorax, and consequently to move the abdomen in relation to the cephalothorax. Three other muscles, again two of them being paired, lie in the pedicel itself, and act as compressors. Other muscles connected to the pedicel lie in the abdomen, and will be considered with the abdominal muscles, as they bring about the movement of the posterior area of the body.

TABLE X  
Muscles of the pedicel

| Muscle                           | No. | Homologue* | Origin  | Insertion  |
|----------------------------------|-----|------------|---|--|
| Dorsal median levator (unpaired) | 73  | —          | Carapace  | Medial area of anterior rim of lorum             |
| Ventral levator                  | 74  | 15A, B     | Dorsal surface of endosternite  | Ventrally and medially on anterior arms of lorum |
| Dorsal levator                   | 75  | —          | Carapace  | Dorsally on anterior arms of lorum               |
| Anterior compressor              | 76  | 72         | Sternite of pedicel   | Medially and ventrally on lateral edge of lorum  |
| Posterior compressor             | 77  | 73         | Posteriorly to 76   | Ventrally on posterior arms of lorum             |
| Lateral compressor (unpaired)    | 78  | 71         | Passes from the medial surface of one anterior arm of the lorum to that of the other, under the mid-gut |  |

\*The homologues shown were described by Brown (7) in *Agelena naevia*.

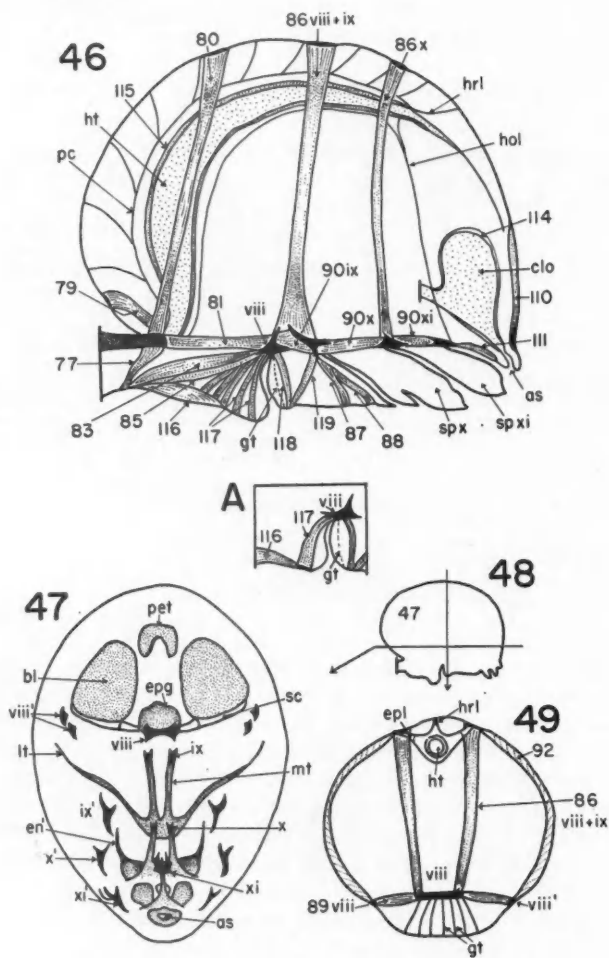
## C. ABDOMEN

### 1. EXOSKELETON

(Figs. 46, 47, 50, 54, 55)

The exoskeleton of the abdomen is less heavily sclerotized than that of the cephalothorax, and shows no pleurites of the 11 segments which make up the abdomen. There are thickenings of the abdominal wall, visible externally, to mark the points of attachment of the larger muscles. The main portion of the abdomen is made up of the segments 8, 9, 10, and 11 of the arachnid body. Because of the ventral position of the appendages, and the reduction of the dorsoventral musculature, the segmentation is best seen in the ventral portions of the abdomen. The eighth segment, which bears the book lungs (*bl*, Fig. 47) and the genital aperture, extends from the sternite of the pedicel to the epigastric furrow. This furrow runs across the ventral wall of the abdomen, and contains the genital aperture medially, and the spiracles of the book lungs laterally. The ninth segment, bearing the median and lateral tracheae (*mt*, *lt*, Fig. 47), is the most extensive ventrally, as it runs from the epigastric furrow to the spiracle of the tracheae, immediately anteriorly to the anterior spinnerets. The 10th segment, bearing the anterior lateral

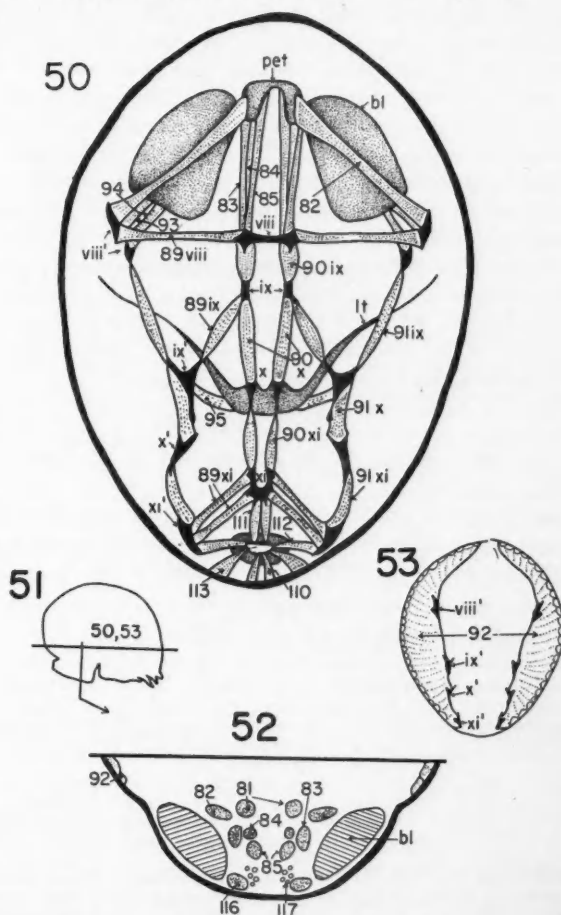
spinnerets (*sp* x, Fig. 54) and the median colulus (*co*, Fig. 54), and the 11th segment, bearing the posterior median and posterior lateral spinnerets (*sp'* xi, *sp* xi, Figs. 54, 55), are quite short, occupying only the areas taken up by the anterior and posterior spinnerets, respectively. The remaining segments, ventrally, are restricted to a small area between the posterior spinnerets and the anus (*as*, Figs. 46, 47).



FIGS. 46-49. Fig. 46. Parasagittal section of abdomen of female. A, parasagittal section of ventral portion of abdomen of male. Fig. 47. Dorsal view of ventral portion of abdomen, as indicated in Fig. 48. Fig. 48. Lateral view of abdomen. Fig. 49. Cross-section of abdomen, as indicated in Fig. 48.

## 2. ENDOSKELETON (Figs. 46, 47, and 50)

The endoskeleton of the abdomen consists of ectodermal apodemes and mesodermal endosternites; the latter rest either on the apodemes or directly on the abdominal wall. The apodemes occur at the posterior limits of each of the four major abdominal segments, while the endosternites are arranged in two longitudinal series, one medial, in which the endosternites rest on the tips of the apodemes, and one lateral, in which they lie in direct contact with the abdominal wall. There is a paired endosternite which does not belong to either series, but is associated with the anterior lateral spinnerets.



FIGS. 50-53. Fig. 50. Dorsal view of ventral portion of abdomen, as indicated in Fig. 51. Fig. 51. Lateral view of abdomen. Fig. 52. Cross-section of ventral portion of abdomen, as indicated in Fig. 51. Fig. 53. Dorsal view of ventral portion of abdomen, as indicated in Fig. 51.

The following structures may be seen in Figs. 47, 50. Median endosternite viii (viii) rests on the dorsal limit of the apodeme forming the epigastric furrow, the two members of the pair being joined by a tendinous band. Median endosternite ix (ix) lies immediately posteriorly to the preceding, on the anterior tip of the median tracheae. Median endosternite x (x) lies similarly on the anterior tip of a forked apodeme arising between the anterior and posterior spinnerets, while median endosternite xi (xi) occurs on the unpaired median apodeme between the posterior spinnerets and the anus. This endosternite is unpaired, apparently as a result of fusion of the members of the pair.

The lateral endosternite viii (viii') consists of a dorsal and a ventral portion, the two being joined by a short tendon. The elements of this endosternite occur laterally to their median counterpart, just posteriorly to the spiracle of the book lungs. Lateral endosternite ix (ix') lies laterally and far posteriorly to its median fellow, beside the lateral trachea of the ninth segment. Lateral endosternites x and xi (x' and xi') lie at the lateral edges of the spinneret area, laterally to their corresponding median endosternites.

The endosternite of the anterior lateral spinneret is situated on the anterolateral corner of the spinneret. This endosternite was not considered to be part of either of the longitudinal series, as it does not give origin to any of the extrinsic muscles of the spinnerets, nor does it have muscular connection with more than one of the other endosternites. It does, however, receive one of the muscles of the anterior lateral spinnerets (102x), and it also gives rise to part of the intrinsic musculature of the spinneret.

### 3. MUSCULATURE

#### (a) *Muscles Causing Movement of the Abdomen on the Pedicel (Figs. 46, 50, and Table XI)*

There are seven paired muscles which originate on the posterior edges of the pedicel, and insert at various points in the abdomen, rendering possible a variety of movements of the abdomen in relation to the pedicel.

TABLE XI  
Muscles causing movement of the abdomen on the pedicel

| Muscle            | No. | Homologue* | Origin   | Insertion  |
|-------------------|-----|------------|--|--|
| Anterior levator  | 79  | 74         | Dorsal surface of posterior arms of lorum of pedicel   | Anterior wall of abdomen, dorsally to posterior arms |
| Posterior levator | 80  | 75         | Posteriorly to 79                                      | Dorsal wall of abdomen, laterally to heart           |
| Ventral levator   | 81  | 77         | Ventrally on posterior tips of posterior arms of lorum | Median endosternite viii                             |
| Lateral levator   | 82  | 76         | Laterally on posterior tips of posterior arms          | Dorsal portion of lateral endosternite viii          |
| Lateral depressor | 83  | 78A        | Sternite of pedicel                                    | Median endosternite viii                             |
| Median depressor  | 84  | 78B        | Medially to 83   | Medially to 83                                       |
| Ventral depressor | 85  | 78C        | Ventrally to 84  | Ventrally to 84                                      |

\*The homologues shown were described by Brown (7) in *Agelena naevia*.

(b) *Muscles Compressing the Abdomen (Figs. 46, 49, 50, 53, and Table XII)*

There is a series of muscles which are segmentally arranged to join the endosternites to one another, and to the abdominal wall, and it is the function of these muscles to decrease the size of the abdominal cavity. There is also a series of unstriated muscle fibers arising from the ventrolateral area of the abdominal wall, at the level of the lateral endosternites, and passing anterad and dorsad along the abdominal wall to the dorsal surface of the abdomen, at a level with the origins of the dorsal compressors of the abdomen. The fibers, which appear to have multiple insertions along their paths, have been named collectively the lateral dorsal compressor (92, Figs. 49, 53). The compressing action of all these muscles supplements direct muscular action on the spinning apparatus, the circulatory system, and the digestive system.

TABLE XII  
Muscles compressing the abdomen

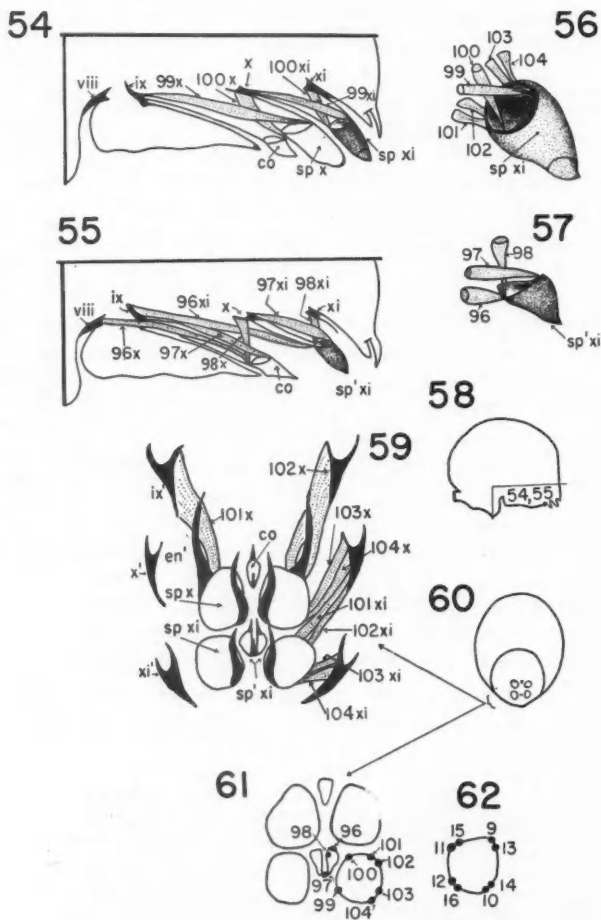
| Muscle                             | No.           | Homologue*          | Origin   | Insertion  |
|------------------------------------|---------------|---------------------|--|--|
| Dorsal compressor viii + ix        | 86viii + ix   | <i>dv</i> viii + ix | Median endosternites viii and ix                           | Dorsal wall of abdomen, laterally to heart                           |
| Dorsal compressor x                | 86x           | <i>dv</i> x         | Median endosternite x                                      | Posteriorly to 86viii + ix   |
| Anterior ventral compressor        | 87            | 12?                 | Median endosternite ix                                     | Ventral wall of abdomen (segment ix)                                 |
| Posterior ventral compressor       | 88            | 13?                 | Median endosternite ix                                     | Posteriorly to 87 (segment ix)                                       |
| Lateral compressor viii            | 89viii        | <i>ml</i> viii      | Median endosternite viii                                   | Dorsal part of lateral endosternite viii                             |
| Lateral compressor ix              | 89ix          | <i>ml</i> ix        | Median endosternite ix                                     | Lateral endosternite ix  |
| Anterior lateral compressor xi     | 89xi <i>a</i> | 84                  | Median endosternite xi                                     | Lateral endosternite xi  |
| Posterior lateral compressor xi    | 89xi <i>p</i> |                     | Posteriorly to 89xi <i>a</i>                               | Posteriorly to 89xi <i>a</i>   |
| Median longitudinal compressor ix  | 90ix          | <i>ml</i> ix        | Median endosternite viii                                   | Median endosternite ix   |
| Median longitudinal compressor x   | 90x           | <i>ml</i> x         | Median endosternite ix                                     | Median endosternite x  |
| Median longitudinal compressor xi  | 90xi          | <i>ml</i> xi        | Median endosternite x                                      | Median endosternite xi   |
| Lateral longitudinal compressor ix | 91ix          | <i>ll</i> ix        | Ventral part of lateral endosternite viii                  | Lateral endosternite ix  |
| Lateral longitudinal compressor x  | 91x           | <i>ll</i> x         | Lateral endosternite ix                                    | Lateral endosternite x   |
| Lateral longitudinal compressor xi | 91xi          | 84?                 | Lateral endosternite x                                     | Lateral endosternite xi  |
| Lateral dorsal compressor          | 92            | (Brown)             | Lateral wall of abdomen, at level of lateral endosternites | Dorsal wall of abdomen, at level of insertions of dorsal compressors |

\*With the exception of the homologues of the anterior and posterior ventral compressors of the abdomen, which are described by Purcell (27) in *Segestria*, the homologues designated by Arabic numerals, together with that of muscle 92, were described by Brown (7) in *Agelena naevia*. The remaining homologues, represented by letters and Roman numerals, were described by Rasmont (29) in *Phoneyssa*, using nomenclature set up by Millot (22).

(c) *Spinnerets*I. *Extrinsic Muscles (Figs. 54-62 and Table XIII)*

The appendages of segments x and xi consist of median and lateral elements. The lateral spinnerets of both segments are large and made up of two segments, while the median spinnerets are smaller and have but one segment. The anterior median spinnerets have become fused, forming the non-functional colulus.

Each of the sets of spinnerets, median and lateral, is operated by identical sets of muscles. Each of the three muscles operating the median spinnerets originates on one of the median endosternites, and although all muscles are paired, some fuse with the other member of the pair before inserting. Each lateral spinneret receives two muscles from the median endosternites and four from the lateral endosternites.



FIGS. 54-62. Fig. 54. Parasagittal section of portion of abdomen, as indicated in Fig. 58 (after removal of posterior median spinneret). Fig. 55. The same, after removal of lateral spinnerets. Fig. 56. Medial view of posterior lateral spinneret. Fig. 57. Medial view of posterior median spinneret. Fig. 58. Parasagittal plan of abdomen. Fig. 59. Dorsal view of portion of abdomen, as indicated in Figs. 58 and 60. Fig. 60. Dorsal view of ventral portion of abdomen. Fig. 61. Spinneret area, as seen from above. Fig. 62. Plan of insertions of extrinsic muscles of coxa of leg.

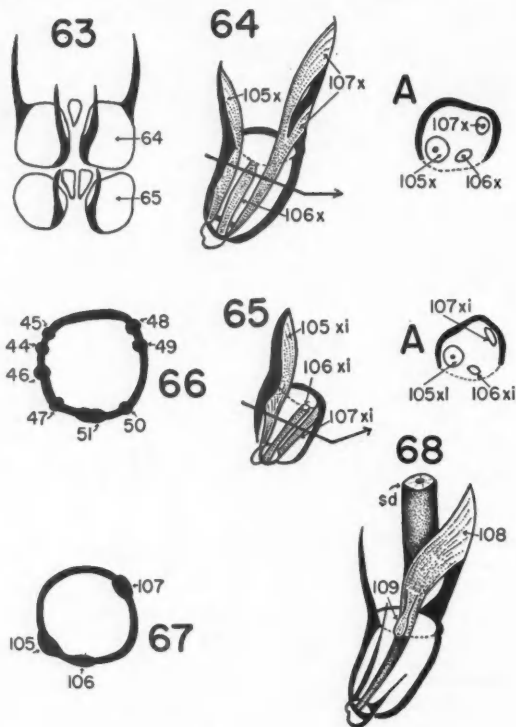
TABLE XIII  
Muscles of the spinnerets

| Muscle                        | No.   | Homologue* | Origin                   | Insertion   |
|-------------------------------|-------|------------|--------------------------|---|
| Colulus                       |       |            |                          |   |
| Ventral depressor x           | 96x   | —          | Median endosternite viii | Medially on proximal ventral rim of colulus (pair fuses)  |
| Levator x                     | 97x   | —          | Median endosternite ix   | Medially on proximal dorsal rim of colulus (pair fuses)   |
| Dorsal depressor x            | 98x   | —          | Median endosternite x    | With 96x (pair fuses)                                     |
| Posterior median spinnerets   |       |            |                          |   |
| Ventral depressor xi          | 96xi  | 89?        | Median endosternite ix   | Medially on proximal ventral rim of spinneret             |
| Levator xi                    | 97xi  | 90?        | Median endosternite x    | Medially on proximal dorsal rim of spinneret (pair fuses) |
| Dorsal depressor x            | 98xi  | —          | Median endosternite xi   | With 96xi   |
| Anterior lateral spinnerets   |       |            |                          |   |
| Levator x                     | 99x   | 86         | Median endosternite ix   | Dorsomedial corner of proximal rim of spinneret           |
| Depressor x                   | 100x  | 85         | Median endosternite x    | Ventromedial corner of proximal rim of spinneret          |
| Anterior median abductor x    | 101x  | 87         | Lateral endosternite ix  | Laterally on proximal ventral rim of spinneret            |
| Anterior lateral abductor x   | 102x  |            | Posteriorly to 101x      | Laterally on endosternite of anterior lateral spinneret   |
| Posterior lateral abductor x  | 103x  | 88         | Lateral endosternite x   | Dorsally on proximal lateral rim of spinneret             |
| Posterior median abductor x   | 104x  |            | Posteriorly to 103x      | Laterally on proximal dorsal rim of spinneret             |
| Posterior lateral spinnerets  |       |            |                          |   |
| Levator xi                    | 99xi  | 94         | Median endosternite x    | Dorsomedial corner of proximal rim of spinneret           |
| Depressor xi                  | 100xi | 93         | Median endosternite xi   | Ventromedial corner of proximal rim of spinneret          |
| Anterior median abductor xi   | 101xi | 95         | Lateral endosternite x   | Laterally on proximal ventral rim of spinneret            |
| Anterior lateral abductor xi  | 102xi |            | Posteriorly to 101xi     | Ventrally on proximal lateral rim of spinneret            |
| Posterior lateral abductor xi | 103xi | 96         | Lateral endosternite xi  | Dorsally on proximal lateral rim of spinneret             |
| Posterior median abductor xi  | 104xi |            | Posteriorly to 103xi     | Laterally on proximal dorsal rim of spinneret             |

\*All homologues shown were described by Brown (7) in *Agelena naevia*.

## II. Intrinsic Muscles (Figs. 63-67 and Table XIV)

The terminal segments of the large lateral spinnerets are operated by three muscles arising on the proximal portions of the basal segment of the spinneret, and inserting on the proximal rim of the terminal segment. The intrinsic musculature of the anterior lateral spinneret is more strongly developed than that of its posterior counterpart, while the median spinnerets, consisting of only one segment, have no intrinsic musculature.



FIGS. 63-68. Fig. 63. Dorsal view of spinneret area. Fig. 64. Posterior view of anterior lateral spinneret, after removal of posterior wall. Fig. 64. A, cross-section of the same, as indicated. Fig. 65. Posterior view of posterior lateral spinneret, after removal of posteral wall. Fig. 65. A, cross-section of the same, as indicated. Fig. 66. Plan of proximal rim of trochanter of leg. Fig. 67. Corresponding plan of proximal rim of terminal segment of lateral spinneret. Fig. 68. Posterior view of anterior lateral spinneret.

### (d) Silk Duct (Fig. 68 and Table XV)

The duct of the ampullate silk gland passes into the anterior lateral spinneret. There are two muscles inserting on the wall of the duct as it enters the spinneret, one from the endosternite of the spinneret, and one from the proximal rim of the appendage. It was not clearly determined whether there was a muscular wall in this portion of the duct, or whether the investing fibers of the dilators simply form a partial sheath.

TABLE XIV  
Intrinsic muscles of the spinnerets

| Muscle   | No.   | Homologue*   | Origin   | Insertion                                |
|--|-------|--------------|--|--|
| Anterior lateral spinnerets (intrinsic muscles)  |       |              |  |  |
| Adductor x                                       | 105x  | { 105<br>108 | Anterior projection of dorsomedial corner of proximal rim of basal segment | Proximal medial rim of terminal segment  |
| Retractor x                                      | 106x  | 107          | Medially on proximal dorsal rim of basal segment                           | Proximal dorsal rim of terminal segment  |
| Abductor x                                       | 107x  | 106          | Medial surface of endosternite of anterior lateral spinneret               | Proximal lateral rim of terminal segment |
| Posterior lateral spinnerets (intrinsic muscles) |       |              |  |  |
| Adductor xi                                      | 105xi | { 101<br>104 | Similarly to 105x  | Similarly to 105x                        |
| Retractor xi                                     | 106xi | 103          | Similarly to 106x  | Similarly to 106x                        |
| Abductor xi                                      | 107xi | 102          | Ventrolateral corner of proximal rim of basal segment                      | Similarly to 107x                        |

\*All homologues shown were described by Brown (7) in *Agelena naevia*.

TABLE XV  
Muscles of the silk duct

| Muscle                           | No. | Homologue* | Origin   | Insertion                                     |
|----------------------------------|-----|------------|--|---|
| Duct of the ampullate silk gland |     |            |  |   |
| Lateral dilator                  | 108 | 110?       | Medial surface of endosternite of anterior lateral spinneret | Ventral, lateral, and dorsal surfaces of duct |
| Posterior dilator                | 109 |            | Proximal dorsal rim of anterior lateral spinneret            | Dorsal surface of duct                        |

\*All homologues shown were described by Brown (7) in *Agelena naevia*.

(e) *Respiratory System (Fig. 50 and Table XVI)*

There are only three muscles, all paired, which relate directly to the organs of respiration in the black widow. These are the two dilators of the book lung spiracles, and the dilator of the tracheal spiracle.

(f) *Anus and Cloaca (Figs. 46, 50, and Table XVI)*

There are four paired dilators of the anal opening, arising from median and lateral endosternites and from the abdominal wall. The cloaca possesses a muscular layer in its wall, made up of apparently unstriated fibers lying longitudinally along the organ, and serving to constrict it.

(g) *Heart (Figs. 46, 49, and Table XVI)*

The heart of the black widow spider is a muscular tube lying dorsomedially in the abdomen, tapering posteriorly to form the posterior aorta, and anteriorly, the anterior aorta, which passes into the cephalothorax through the pedicel. The pericardium (*pc*, Fig. 46), a loose sac of connective tissue surrounding the heart, is suspended by a series of cardiac ligaments. The hypercardiac ligaments (*hrl*, Fig. 46) lie in the median line and connect the pericardium to the dorsal surface of the abdomen. The epicardiac ligaments

TABLE XVI

Abdominal muscles of the respiratory, digestive, circulatory, and reproductive systems

| Muscle                   | No. | Homologue* | Origin   | Insertion   |
|--------------------------|-----|------------|--|---|
| Spiracle of book lung    |     |            |  |   |
| Dorsal dilator           | 93  | 11         | Dorsal part of lateral endosternite viii                                     | Posterior lip of spiracle   |
| Ventral dilator          | 94  |            | Ventral part of lateral endosternite viii                                    | Medially to 93  |
| Spiracle of tracheae     |     |            |  |   |
| Lateral dilator          | 95  | —          | Lateral endosternite ix  | Lateral wall of lateral tracheae and lateral lip of spiracle                |
| Anus                     |     |            |  |   |
| Dorsal dilator           | 110 | —          | Posterior wall of abdomen, dorsally to anus                                  | Medially on dorsal anal lobe  |
| Ventral dilator          | 111 | 98         | Median endosternite xi   | Medially on ventral anal lobe   |
| Lateral dilator          | 112 | 97         | Lateral endosternite xi  | Laterally to anus   |
| Oblique dilator          | 113 | —          | Posterior wall of abdomen, laterally to 110                                  | Between 110 and 112   |
| Cloaca                   |     |            |  |   |
| Constrictor (unpaired)   | 114 | (Millot)   | Unstriated fibers running longitudinally in wall of cloaca                   |   |
| Heart                    |     |            |  |   |
| Constrictor (unpaired)   | 115 | (Millot)   | Unstriated fibers in an outer longitudinal layer and an inner circular layer |   |
| Genital aperture         |     |            |  |   |
| Ventral anterior dilator | 116 | 5          | Sternite of pedicel  | Anterolateral rim of aperture and, in the female, anterior edge of epigynum |
| Dorsal anterior dilator  | 117 | 4          | Median endosternite viii   | Medially to 116 (separate fibers in female)                                 |
| Posterior dilator viii   | 118 | 16         | Median endosternite viii   | Posterior rim of aperture   |
| Posterior dilator ix     | 119 | 17         | Median endosternite ix   | With 118  |

\*With the exception of the homologues of the constrictors of the heart and the cloaca, which were described by Millot (23), and those of the ventral and lateral dilators of the anus, which were described by Brown (7) in *Agelena naevia*, the homologues shown were described by Furcell (28) and Kästner (14) in *Segestria*.

(*epl*, Fig. 49) also suspend the pericardium dorsally, but are directed slightly laterad as well, to be attached to the dorsal wall of the abdomen at a level with the points of origin of the dorsal compressors of the abdomen. The hypocardiac ligaments (*hol*, Fig. 46), of which there is only one pair, pass from the ventral surface of the pericardium, along the anterior surface of the cloaca, and insert on median endosternite xi.

(*h*) *Genital Aperture* (Figs. 46, 46a, 49, and Table XVI)

The genital aperture of the female black widow is dilated by four pairs of muscles, two of which are situated anteriorly to the aperture, and two posteriorly to it. There is a group of genital tendons (*gt*, Figs. 46, 49) lying in the transverse plane, and extending from the median endosternite viii to the posterior rim of the genital aperture. They do not appear to be contractile in nature.

The muscles dilating the genital aperture of the male are almost identical with those of the female, with two minor exceptions. The ventral anterior dilator (116) of the male inserts entirely on the anterior rim of the aperture, and the dorsal anterior dilator (117) is narrower than in the female, the fibers lying in one bundle.

### Discussion and Summary

In the preceding account of the musculature of *Latrodectus mactans*, there were 537 muscles described in the female, and 533 in the male, comprising parts of the locomotive, digestive, reproductive, respiratory, circulatory, and excretory systems, and of the poison and silk mechanisms. In spite of the fusion of the segments of the arachnid body to form the cephalothorax, pedicel, and abdomen, and the modification of appendages as part of the process of specialization, the musculature exhibits a strong segmentation. It is seen in the cephalothorax, where the extrinsic muscles of the appendages are segmentally arranged, although the fusion of the body segments has caused the longitudinal and lateral segmental muscles to disappear. It is seen also in the abdomen, where, conversely, there is a reduction of the musculature of the appendages, but the longitudinal and lateral segmental muscles partially persist.

When considering the functions of muscles in the body of a spider, it is important to bear in mind that no muscle with two points of attachment has but one function. Although the main action will occur at the point designated as the insertion, there is a secondary action at the point of origin. For example, the muscles passing from the cephalothoracic endosternite to the appendages exert their principal action on the basal segments of the appendages. At the same time, they act to maintain the position of the endosternite in the center of the cephalothoracic cavity, or, depending on which muscles are contracting simultaneously, to alter the position of the endosternite. The latter action would subtly change the exact functions of the other muscles attached to the endosternite, by altering the angles with which they insert on the various structures. Similarly, the contraction of

the muscles passing from the carapace to the appendages will not only move the appendages directly, but will have an indirect effect in compressing the carapace, and therefore, of increasing the pressure within the cephalothorax and thereby facilitating the extension of the terminal segments of the appendages by means of increased blood pressure. The entire system of muscles, then, may be regarded in one sense as a complex unit capable of countless variations in function, enabling the spider to carry on a highly specialized existence.

The study of the muscular system of a spider reveals that only recently has it been possible to carry out valid comparative studies. While the earlier workers achieved a general understanding of the arrangement of muscles in the araneid body, their interpretations of detail sometimes suffered because of lack of knowledge of araneid morphology at the time of their research. Within recent years, however, such workers as Kästner (15) and Millot (23) have extended knowledge of araneid morphology to set the scene for further comparative studies of spider musculature, particularly from the viewpoint of segmentation. While it is not the purpose of the present work to set forth such a study, it is of value to compare briefly the disposition of muscles in the black widow with what has been described by other authors for various spiders. Tables I to XVI indicate those descriptions which correspond most closely with the musculature of *Latrodectus mactans*.

In the cephalothorax there has been a fusion of endoskeletal as well as exoskeletal elements. The result is the large, single endosternite which, in the black widow, is almost identical with that described by Millot (23) in *Lycosa*, as are the dorsal suspensors. The anterior and posterior muscles suspending the endosternite are as described by Brown (7).

The extrinsic muscles of the cephalothoracic appendages were recognized by Kessler (16) to consist of a group originating on the carapace, and one on the endosternite. The muscles were well described by Brown (7), and by Steinbach (36), the latter author discovering the posterior rotator of the palpi and first two legs (called the lateral rotator of the palpi in the present work). Steinbach's system of homology between the muscles of the chelicerae and those of the legs has been followed here. The extrinsic muscles of the appendages of the black widow were found also to be as described by Steinbach for several species, with the exception of the insertions and proposed functions of the muscles of the palpal coxae. The homologies, however, were retained.

The muscles of the cephalothoracic alimentary canal are as in the account given by Brown (7), although the dilators of the mid-gut have not previously been described by any of the authors who have mentioned the various muscles related to this system.

The compressor of the carapace has been mentioned only by Brown (7) in spiders, although similar muscles occur in the cephalothorax of the Pedipalpi, as noted by Börner (6).

Muscles of the coxal gland have not been previously described in spiders although two muscular dilators were observed by Börner (6) in the Pedipalpi,

a group of arachnids considered to be most closely related to spiders. The extreme attenuation of these striated fibers may be responsible for their being overlooked, if they are present generally in spiders.

The longitudinal muscular constrictor of the venom gland is made up of only one layer of striated fibers, as reported by Reese (30) in the black widow and by Kessler (16) in *Lycosa*. The reflection of the fibers on a chitinous ring in the gland was not found, although reported in *Latrodectus* by Ancana (1) and by Porto (26).

The intrinsic musculature of the walking legs is difficult to interpret accurately, because as pointed out by Bonnet (4) the muscular fibers are tightly packed within the slender segments of the legs, making the matter of separating the fibers into bundles rather doubtful. Petrunkevitch, quoted by Brown (7), described 15 muscles in the leg, while Dillon (10), on the other hand, mentioned 31 muscles. The intrinsic leg musculature of the black widow seems to correspond most closely with the description of *Eurypelma* given by Snodgrass (35). Because the patella and possibly the basitarsus lack extensor muscles, and yet the dicondylic joints are capable of extension, it is highly probable that the effects of blood pressure are brought into use to achieve extension. This was suggested by such early workers as Gaubert (13), and Ellis (11) has proposed a mechanism for the process.

The intrinsic musculature of the palpi has not previously been described in published accounts, to the knowledge of the authors, although it was implicit in the work of such authors as Brown (7) that the musculature was identical with that of the walking legs. In *Latrodectus mactans*, the palp of the female is found to lack four of the muscles found in the leg, and the palp of the male, six. This was the only substantial difference found in the muscles of the two sexes, and is easily correlated with the extreme modification of the male palp for purposes of copulation.

The cheliceral musculature is the same as that described by Schimkewitsch (33) in *Epeira*, and by Gaubert (13) in several species, although the latter author believed that the three flexors should be considered merely as branches of one muscle.

The seventh segment of the body, the pedicel, has received relatively little attention. The nature of the exoskeleton seems to have been inadequately described, but Brown (7) gives a complete account of the musculature, lacking only the two dorsal levators found in the black widow. Petrunkevitch (25) states that the two dorsoventral compressors within the pedicel are found in all spiders. A muscle described in other species by Kästner (14) and by Rasmont (29) as passing from the cephalothoracic endosternite posterad through the pedicel to median endosternite viii of the abdomen was not found.

Table XVII offers proposed homologies between muscles of the posterior part of the seventh segment, and those of the abdominal segments. The scheme suggests that muscle 81 might be homologous with the dorsoventral muscle 86. To make this possible, however, either the muscle designated

86viii+ix would have to be considered as 86ix, in spite of its connection with median endosternite viii, or the dorsoventral muscle of the eighth segment would have to be considered to have divided into two muscles, 81 and part of 86viii+ix.

TABLE XVII

Homologies between posterior muscles of the pedicel and those of the abdomen

| Muscle of pedicel    | Muscle of abdomen |
|----------------------|-------------------|
| 76 (Fig. 42)         | 92 (Fig. 53)      |
| 77 (Fig. 42)         | 86 (Fig. 46)      |
| (83,84,85) (Fig. 50) | 90 (Fig. 50)      |
| 82 (Fig. 50)         | 91 (Fig. 50)      |
| 78 (Fig. 44)         | 89 (Fig. 50)      |

The two muscles 79 and 80 (Fig. 46) probably represent remnants of a median longitudinal series of muscles, segmentally arranged, and observed by Millot (23) in the adult of *Liphistius*, by Purcell (27) in immature *Segestria*, and by the present authors in late embryonic black widow spiders. No trace of this series of muscles was found in the adult spider.

The musculature of the abdomen has been most widely studied, because the arrangement of the muscles supports the idea of the persistence of segmentation in spiders. Millot (22) described a series of araneomorphic abdomens from this point of view, and was followed by Rasmont (29), who described the abdominal musculature of two mygalomorphic species of spiders. These papers are valuable in presenting the general picture of segmental arrangement, but they are lacking in details of the musculature of the abdominal appendages, and they do not seem to recognize the relationships existing between the ectodermal apodemes and the mesodermal endosternites. This relationship was first pointed out by Purcell (27). Brown (7), too, refers to the abdominal endosternites as "floating tendons".

Several authors have made mention of the "abdominal sac" encasing the abdomen. In *Latrodectus mactans* this was found to consist of unstriated fibers which do not encircle the abdomen, but are found only laterally. They have been considered to be the remnants of segmentally arranged muscles connecting the sternites and tergites of the primitive arachnid abdomen. Such muscles are found in the Pedipalpi, according to Börner (6), with additional points of attachment on the arthrodial membrane between the sclerites.

It was formerly believed that most of the muscles in the anterior portion of the abdomen were related to the organs of respiration. Such a hypothesis was presented by Weiss (39). Kästner (14), however, showed that the muscles in question do not occur with the second pair of book lungs when a posterior pair is present, and yet when the anterior pair of book lungs is lacking, the muscles, with the exception of the homologues of muscles 93

and 94, persist. Therefore, Kästner suggested that the muscles in the anterior part of the abdomen serve to move the abdomen on the pedicel, and such an interpretation was used in the description of the muscles of the black widow.

The dilator of the lateral trachea of segment ix has not been described as such, but Millot (22) notes a muscle related to the posterior pair of book lungs in *Liphistius*, to which the tracheal muscle could be homologous. It is considered that the lateral tracheae are derivatives of the book lungs, and that both structures are modified abdominal appendages.

TABLE XVIII

Homologies between muscles of the spinnerets  
and those of the cephalothoracic appendages

| Muscle of spinneret        | Muscle of cephalothoracic appendage |
|----------------------------|-------------------------------------|
| Extrinsic muscles          |                                     |
| 101 (Fig. 59)              | 9                                   |
| 104 (Figs. 56 and 59)      | 10                                  |
| (96, 98) (Figs. 55 and 57) | 11                                  |
| 97 (Figs. 55 and 57)       | 12 (Figs. 8, 11, 12, and 13)        |
| 102 (Figs. 56 and 59)      | 13                                  |
| 103 (Figs. 56 and 59)      | 14                                  |
| 100 (Figs. 54 and 56)      | 15                                  |
| 99 (Figs. 54 and 56)       | 16                                  |
| Intrinsic muscles          |                                     |
| 105 (Figs. 64 and 65)      | (44,46,47,45) (Fig. 22)             |
| 106 (Figs. 64 and 65)      | (50,51) (Figs. 22B and C)           |
| 107 (Figs. 64 and 65)      | (48,49) (Figs. 22 and 32)           |

The musculature of the spinnerets has been described by Brown (7), Purcell (28), and Rasmont (29). These workers recorded relatively few muscles operating the spinnerets. Brown (8) proposed a system of homology between the extrinsic muscles of the spinnerets and those of the coxae of the legs. Although it was recognized by Brown that the lateral and median spinnerets arise by a division of the paired abdominal appendages of segments x and xi (the divisions being considered by some to be equivalent to the exopodite and endopodite of the Crustacean appendages), he has suggested that the four muscles found by him to operate the lateral spinnerets are homologous with the four muscles passing from the cephalothoracic endosternite to the coxae of the legs, and that the three muscles operating the posterior median spinnerets are also homologous to these muscles. It would seem more logical to assume that the muscles operating the lateral and median spinnerets are together homologous with all the muscles of one coxa. Such an assumption has been made in the homologies suggested in Table XVIII and illustrated in Figs. 61 and 62.

The muscles found in the black widow spider to operate the colulus have not been previously described, although Millot (31) established that this small median lobe represented the fusion of the anterior median spinnerets, which are no longer functional.

Brown (8) also set up homologies between the muscles of the terminal segment of the lateral spinneret, and those of the trochanter of the leg. He found four muscles in each case. There are three muscles operating the terminal segment of the lateral spinneret in the black widow, and three major groups of muscles acting on the trochanter, both sets inserting on all but the anterior rim of the distal segments. The homologies proposed for the black widow may be found in Table XVIII and in Figs. 66 and 67. It should be noted that whereas the proposals made by Brown were based on examination of both adult and embryonic material, the homologies suggested for *Latrodectus mactans* arise from a study of adult spiders only.

Leydig (20) mentioned muscles associated with the silk glands, but otherwise no description has been given of the two dilators of the duct of the ampullate silk gland in the black widow. Brown (7) described a muscle passing from the dorsal to the ventral rim of the anterior lateral spinneret. It is possible that this muscle represents the two dilators found in the black widow, since it would not be difficult to interpret the situation as the duct passing through a muscle originating on the endosternite of the spinneret and inserting on the rim of the appendage. However, because this endosternite has been considered to be related to the spinneret, and not to belong to one of the longitudinal series, such an interpretation was not taken. The fact that part of the silk duct receives muscles from the base of an appendage, and that the duct itself possesses a sclerotized intima in the form of a slender tube near its distal end, suggests a relationship between the silk glands and the coxal glands of the cephalothorax. This theory has been mentioned by Snodgrass (35). Of the authors who have described muscles serving to dilate the anal opening, Millot (22), Brown (7), and Rasmont (29), none has mentioned more than two muscles, as compared with the four found in *Latrodectus mactans*.

The heart of the black widow spider is typical of that of other species described by Millot (22) and Rasmont (29). The presence of only one pair of hypocardiac ligaments is an indication of the reduction of abdominal morphological characteristics.

Muscles of the genital aperture have been described by such workers as Brown (7) and Rasmont (29), but the muscles in the black widow were found to be identical with those described by Purcell (28) and Kästner (14). Slight differences between the sexes, confined to shape and points of insertion of two muscles, are probably related to the relative simplicity of the male aperture.

The number of muscles contained within the cephalothorax of the adult female *Latrodectus mactans* is 78 paired and 6 unpaired; in the cephalothoracic appendages, 125 paired; in the pedicel, 2 paired and 1 unpaired; in the abdomen, 53 paired and 2 unpaired; in the abdominal appendages 6 paired muscles. This makes a total of 264 paired muscles and 9 unpaired, or 537 separate muscles in all.

The adult male lacks 2 of the paired muscles of the palpi, and therefore possesses a total of 262 paired muscles and 9 unpaired, or 533 separate muscles.

### Acknowledgments

This investigation was supported financially by the National Research Council of Canada.

The authors wish to express their gratitude to Dr. L. G. Saunders, Professor of Biology at the University of Saskatchewan, for his helpful suggestions regarding sectioning techniques, and to Mr. Norman Ferrier for his invaluable assistance with the photography.

### References

Those references marked (\*) were unfortunately not available to the authors. Most of the references are listed by Bonnet (5).

- \*1. ANCANA, L. Anatomía e histología del aparato venenoso de *Latrodectus mactans* o Araña capulina. Ann. Inst. Biol., Chapultepec, **2**, 77-84 (1931).
2. BLANCHARD, E. L'organisation du Règne animal. Arachnides. Chez Victor Masson, Paris. 1851-1864.
- \*3. BOETTCHER. Ueber den anatomischen Bau der Kreuzspinne. Programm der höhern Bürgerschule zu Grandenz, 1-17 (1850).
4. BONNET, P. La mue, l'autotomie et la régénération chez les Araignées avec une étude des Dolomèdes d'Europe. Bull. soc. hist. nat. Toulouse, **59**, 237-700 (1930).
5. BONNET, P. Bibliographia Araneorum. Toulouse. 1945.
6. BORNER, C. Beiträge zur Morphologie der Arthropoden. I. Ein Beitrag zur Kenntnis der Pedipalpen. Zoologica, **42**, 1-174 (1904).
7. BROWN, R. B. The musculature of *Agelena naevia*. J. Morphol. and Physiol. **64**, 115-166 (1939).
8. BROWN, R. B. Spinneret muscle homologies in spiders. Trans. Conn. Acad. Arts Sci. **36**, 245-248 (1945).
- \*9. CAPORACCIO, L. DI Il sistema degli Araneidi. Arch. zool. ital., Suppl., Attual. zool. **4**, 35-155 (1938).
10. DILLON, L. S. The myology of the araneid leg. J. Morphol. and Physiol. **90**, 467-480 (1952).
11. ELLIS, C. H. Mechanisms of extension in the legs of spiders. Biol. Bull. **86**, 41-50 (1944).
- \*12. FREY, H. and LEUCKART, R. Lehrbuch der Anatomie der wirbellosen Thiere. Leipzig. 1847.
13. GAUBERT, P. Recherches sur les organes des sens et sur les systèmes tégumentaire, glandulaire et musculaire des appendices des arachnides. Ann. sci. nat. Zool. **13**, 31-184 (1892).
14. KÄSTNER, A. Bau und Funktion der Fächertracheen einiger Spinnen. Z. Morph. Okol. der Tiere, **13**, 463-558 (1929).
15. KÄSTNER, A. and GERHARDT, U. Araneae. Kükenthal-Krumbach's Handbuch der Zoologie, **3**, 497-656 (1938).
16. KESSLER, G. Beitrag zur Naturgeschichte und Anatomie der Gattung *Lycosa*. Bull. Soc. Imp. nat. Moscou, **22**, 480-521 (1849).
- \*17. KOLOSARY, G. Morfológiai Apróságok a Szongáriai Cselőpókról (Beiträge zur Morphologie von *Trochosa singoriensis*). Allat. Kozlem, **25**, 59-65 (1928).
- \*18. LEBERT, H. Die Spinnen der Schweiz, ihr Bau, ihr Leben, ihre systematische Uebersicht. N. Denkschr. schweiz. naturforsch. Ges. **23**, 1-321 (1877).
- \*19. LENDL, A. A Pókok harántezikolt izomrostjainak szövétani szerkezete. (Sur la musculature des Araignées) Math. Termés. Ert. **34**, 258-265 (1916).
- \*20. LEYDIG, F. Zum feineren Bau der Arthropoden. Arch. Anat. Physiol. 376-480 (1855).
21. MILLOT, J. Colulus et filières non fonctionnelles chez les Aranéides. Compt. rend. **190**, 209-210 (1930).
22. MILLOT, J. Métamérisation et musculature abdominale chez les Aranéomorphes. Bull. soc. zool. France, **61**, 181-204 (1936).
23. MILLOT, J. Araneae: Grassé's Traité de zoologie, anatomie, systématique, biologie. VI. Paris. 1949.
24. NIELSEN, E. The biology of spiders. Vol. I. Copenhagen. 1932. pp. 1-248.
25. PETRUNKEVITCH, A. An inquiry into the natural classification of spiders, based on a study of their internal anatomy. Trans. Conn. Acad. Arts Sci. **31**, 299-389 (1933).
26. PORTO, J. Structure of the venomous apparatus of spiders of the genus *Latrodectus*. Soc. Arg. Biol. Rev. **18**, 346-350 (1942).
27. PURCELL, W. F. The development and origin of the respiratory organs in the Araneae. Quart. J. Microscop. Sci. **54**, 1-110 (1909).

28. PURCELL, W.F. The phylogeny of the tracheae in Araneae. *Quart. J. Microscop. Sci.* **54**, 519-563 (1910).
29. RASMONT, R. Myologie abdominale d'une Theraphoside et d'une Dipluride (Araneae, Mygalomorphae). *Bull. soc. zool. France*, **77**, 121-156 (1952).
30. REESE, A. M. The anatomy of the venom glands in the black widow spider, *Latrodectus mactans*. *Trans. Am. Microscop. Soc.* **43**, 170-174 (1944).
31. REMPEL, J. G. The embryology of the black widow spider, *Latrodectus mactans* (Fabr.). *Can. J. Zool.* **35**, 35-74 (1957).
32. SAVORY, T. H. The biology of spiders. London. 1928.
33. SCHIMKEWITSCH, W. Étude sur l'anatomie de l'Epeire. *Ann. sci. nat. Zool.* **17**, 1-94 (1884).
- \*34. SIEBOLD, C. T. VON. *Lehrbuch der vergleichenden Anatomie der wirbellosen Thiere.* Berlin, 506-554 (1848).
35. SNODGRASS, R. E. A textbook of arthropod anatomy. Comstock Publishing Co., Inc., New York, N.Y. 1952.
36. STEINBACH, G. Vergleichende Untersuchungen zur Cheliceren-muskulatur einiger Araneen. *Wiss. Z. Humboldt-Univ., Berlin Math. Naturwiss. Reihe*, **2** (1954).
37. TREVIRANUS, G. R. Über den inneren Bau der Arachniden. *Nürnberg*, **4** (1812).
38. WASMANN, A. Beiträge zur Anatomie der Spinnen. *Abhandl. Ver. Hamburg*, **1**, 131-161 (1846).
39. WEISS, S. Untersuchungen über die Lunge und die Atmung der Spinnen. *Zool. Jahrb.* **39**, 535-545 (1923).

### Explanation of Lettering on Figures

|            |                                      |               |                              |
|------------|--------------------------------------|---------------|------------------------------|
| <i>ab</i>  | abdomen                              | <i>pcm</i>    | paracymbium                  |
| <i>an</i>  | abdominal nerve                      | <i>pes</i>    | sternite of pedicel          |
| <i>ao</i>  | aorta                                | <i>pet</i>    | tergite of pedicel (lorum)   |
| <i>ap</i>  | anterior apodeme                     | <i>ph</i>     | pharynx                      |
| <i>as</i>  | anus                                 | <i>pl</i>     | pretarsus                    |
| <i>ba</i>  | sclerotized bar in arthroal membrane | <i>pla</i>    | patella                      |
| <i>bl</i>  | book lung                            | <i>sa</i>     | sacculus of coxal gland      |
| <i>bt</i>  | basitarsus                           | <i>sc</i>     | spirale of book lung         |
| <i>ca</i>  | carapace                             | <i>sd</i>     | duct of ampullate silk gland |
| <i>ch</i>  | chelicera                            | <i>sg</i>     | subesophageal ganglion       |
| <i>clo</i> | cloaca                               | <i>sp x</i>   | anterior lateral spinneret   |
| <i>cm</i>  | cymbium                              | <i>sp xi</i>  | posterior lateral spinneret  |
| <i>co</i>  | colulus                              | <i>sp' xi</i> | posterior median spinneret   |
| <i>cx</i>  | coxa                                 | <i>ss</i>     | sucking stomach              |
| <i>do</i>  | doublure                             | <i>st</i>     | sternum                      |
| <i>em</i>  | embolus                              | <i>ta</i>     | tarsus                       |
| <i>en</i>  | endosternite of cephalothorax        | <i>tb</i>     | tibia                        |
| <i>ep</i>  | epistome                             | <i>tr</i>     | trochanter                   |
| <i>epg</i> | epigynum                             | <i>tt</i>     | telotarsus                   |
| <i>epl</i> | epicardiac ligament                  | <i>vd</i>     | venom duct                   |
| <i>es</i>  | esophagus                            | <i>vg</i>     | venom gland                  |
| <i>fe</i>  | femur                                | <i>i</i>      | leg i                        |
| <i>gl</i>  | genital tendons                      | <i>ii</i>     | leg ii                       |
| <i>hol</i> | hypocardiac ligament                 | <i>iii</i>    | leg iii                      |
| <i>hrl</i> | hypercardiac ligament                | <i>iv</i>     | leg iv                       |
| <i>ht</i>  | heart                                | <i>viii</i>   | median endosternite viii     |
| <i>la</i>  | labyrinth of coxal gland             | <i>ix</i>     | median endosternite ix       |
| <i>lb</i>  | labrum                               | <i>x</i>      | median endosternite x        |
| <i>lp</i>  | lower lip of sternum                 | <i>xi</i>     | median endosternite xi       |
| <i>lt</i>  | lateral trachea                      | <i>viii'</i>  | lateral endosternite viii    |
| <i>mg</i>  | mid-gut                              | <i>ix'</i>    | lateral endosternite ix      |
| <i>mt</i>  | median trachea                       | <i>x'</i>     | lateral endosternite x       |
| <i>pa</i>  | palpus                               | <i>xi'</i>    | lateral endosternite xi      |
| <i>pc</i>  | pericardium                          |               |                              |

## THE ORIGIN OF TWO GLACIAL RELICT CRUSTACEANS IN NORTH AMERICA, AS RELATED TO PLEISTOCENE GLACIATION<sup>1</sup>

KARL E. RICKER

### Abstract

In North America *Mysis relicta* and *Pontoporeia affinis* occur almost exclusively in basins which were part of a proglacial lake during late glacial time. They also occur in arctic brackish waters, and arctic stocks can have served as the origin of all inland continental stocks provided the ice advanced originally from the northeast across such shallow seas as Foxe Basin and Hudson Bay—in the manner envisaged by Flint's recent hypothesis of how the continental ice sheet was established. On this view, the future relicts were carried across the continent in proglacial lakes as the ice advanced, as far as the Waterton Lakes in Alberta—Montana and the Finger Lakes in New York; during the retreat of the ice, they followed its margin back in a similar series of lakes, remaining in the survivors of the latter whenever they are deep and cold enough.

### Introduction

It has been recognized for many years that the Pleistocene glaciation in North America played an important role in the distribution of plants and animals. Carpenter (9) observed: "The ice sheet, spreading death and desolation before it in its progress southward, yet, in its retreat, induced an extension in the range of species, an adaptation in physiology and habit, and a formation of new local types, which must induce us to regard it as, on the whole, a progressive rather than a retrogressive force".

Two theories with many variations have been advanced to explain the distribution of aquatic glacial relicts: (1) the relicts occupied ocean basins, subsequent elevation of the land brought the basins above sea level, and inflowing water gradually changed them to lakes; (2) the relicts were pushed ahead of the ice, during the glacial period, from salt water into lakes along the ice margin, in whose remnants they survive. Segerstråle (62), Thienemann (72), and Ekman (18-20) have given proofs of the second theory as applied to northern Europe, but in North America there has been no agreement on a hypothesis to explain relict distribution. This paper summarizes information pertinent to discussion of the distribution of two common glacial relicts in North America: the mysidacean *Mysis relicta* Lovén and the amphipod *Pontoporeia affinis* Lindström.

### Distribution

*Pontoporeia affinis* has a circumpolar distribution in brackish portions of northern seas, and also occurs in deep lakes of the glaciated portions of North America and Eurasia. Gurjanova (27) gives records from the following zoogeographical provinces: Chukchi-American, North Atlantic (including

<sup>1</sup>Manuscript received March 11, 1959.

Contribution from the Institute of Fisheries, The University of British Columbia, Vancouver 8, B.C.

the Baltic), Siberian, and White Sea-Spitsbergen. The fresh-water distribution is summarized by Segerstråle (61). A marine relative of *affinis*, *P. femorata* Krøyer, also has a circumpolar arctic distribution, south to New England, the middle Norwegian coast, and the Sea of Japan; and it also occurs in the Kattegat and saltier parts of the Baltic Sea.

Until recently *Mysis relicta* was considered to be a fresh-water representative, or more often a subspecies, of the circumpolar marine species *M. oculata* (Fabricius). More lately other closely related species have been described; their distribution, following Holmquist (32), is as follows: *M. litoralis* (Banner) is circumpolar in the arctic and has a single record, the type locality, in Puget Sound; *M. polaris* Holmquist is known from only two specimens, taken in widely separated arctic localities. *M. gaspensis* Tattersal (southern Labrador and Gulf of St. Lawrence) and *M. mixta* Lilljeborg (North Atlantic and adjacent arctic coasts) also seem very close, though assigned to a different subgenus by Holmquist. In addition there are four species from the southern Caspian Sea. Of the northern species, *polaris* and *oculata* are known from marine and brackish habitats, *litoralis* and *gaspensis* from marine, brackish, or (rarely) fresh waters, and *relicta* from marine (rarely), brackish, and fresh waters. Only *relicta* is found in fresh waters remote from the sea.

Holmquist (31, 32) has shown that a number of former records of *relicta* can be eliminated because of redefinition, or misidentification. *M. relicta* reports from beyond the glacial border in Europe, at Venice, Trieste, and in Herzegovina, are referable to *Diamysis bahirensis* (G. O. Sars) and *Paramysis helleri* (G. O. Sars). *M. relicta* reported from fresh water at Indian Harbour, Labrador, by Rathbun (50), is referred to *M. gaspensis*. Specimens of *relicta* recorded from Bernard Harbour, N. W. T., by Schmitt (60) and Johansen (35) could not be traced by Holmquist, but other collections from there show only *litoralis* and *oculata*.

As in the old world, *Pontoporeia affinis* and *Mysis relicta* in North America are usually found in lakes which are remnants of Pleistocene proglacial lakes (Fig. 1)—that is, lakes which had the continental ice sheet as one of their margins. The lists below include all North American bodies of water for

FIG. 1. Glaciation map of North America, showing glacial relict locations. (Map after Wilson *et al.* (75) and Flint *et al.* (25).)

|       |  |       |                                 |
|-------|--|-------|---------------------------------|
| A     | Cordilleran Ice Sheet  | Biiib | Parc des Laurentides Ice Centre |
| Bi    | Baffin Island, Ellesmere Island,<br>Devon Island and Bylot Island<br>Ice Centres | Biiic | Shickshock Mountains Ice Centre |
| Bii   | Labrador Ice Centre  | C     | "Keewatin Ice Centre"           |
| Biiia | Newfoundland Ice Centre  | D     | Hudson Strait                   |
|       |  | E     | Foxe Basin                      |

Numbered lakes contain either *Mysis relicta* or *Pontoporeia affinis*, or both, as shown on pages 874 and 875.

Dotted areas indicate the extent of the larger Pleistocene proglacial lakes.

The eastern limit of the Cordilleran Ice Sheet is indicated by a line hatched on its left side; the western limit of the Laurentide ("Keewatin") Ice Sheet is indicated by a line hatched on its right side. These two boundaries overlap in places. The southern margin of Pleistocene continental glaciation is indicated by a double-hatched line.

Heavy broken lines indicate glacial melt-water channels.

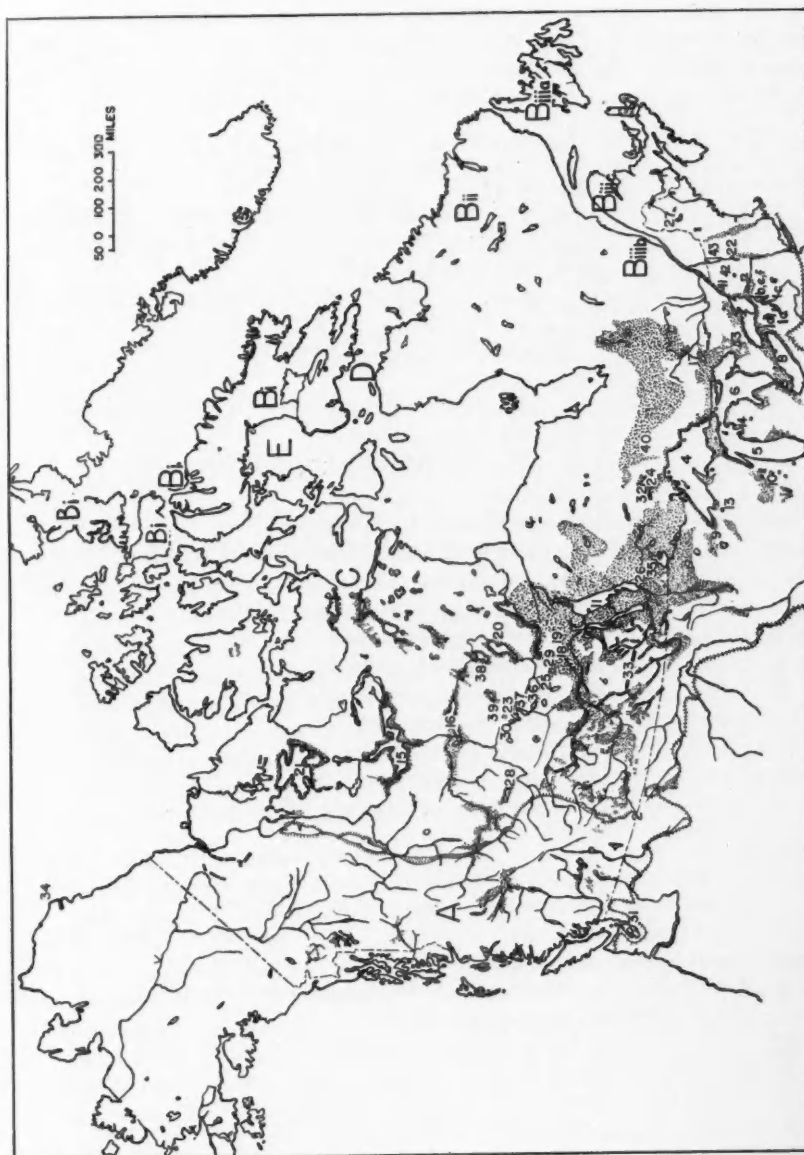


Fig. 1.

which records of *Pontoporeia affinis* and *Mysis relicta* have come to my attention. Investigators quoted for each locality are, however, not necessarily the first or the only ones to record the species from the body of water in question. Some New York lake records, given simply as *Mysis*, are assumed to be *M. relicta*. For all *Mysis* in salt or brackish water, localities are cited only when specimens have been checked by Holmquist (32).

#### FRESH-WATER OCCURRENCES

##### *Mysis relicta* and *Pontoporeia affinis*

1. Finger Lakes, New York—Birge and Juday (4,5); Eaton (15): (a) Keuka Lake; (b) Cayuga Lake; (c) Seneca Lake; (d) Canandaigua Lake; (e) Owasco Lake; (f) Skaneateles Lake
2. Waterton Lakes, Alberta—Rawson (52), Cuerrier and Schultz (12)
4. Lake Superior—Smith (65), Holmquist (32)
5. Lake Michigan—Smith (65), Eggleton (16)
6. Lake Huron—Huntsman (34)
7. Lake Ontario—Huntsman (34), Sibley (64)
8. Lake Erie—Wilson (74), Langlois (39)
10. Green Lake, Green Lake Co., Wisconsin—Marsh (43)
11. Lake Winnipeg, Manitoba—Bajkov (2)
15. Great Slave Lake, Mackenzie—Larkin (41)
16. Lake Athabasca, Saskatchewan and Alberta—Larkin (41)
18. Lake Athapapuskow, Manitoba—Stewart-Hay (70)
19. Second Cranberry Lake, Manitoba—Stewart-Hay (70)
20. Reindeer Lake, Saskatchewan—Rawson (53)
21. Great Bear Lake, Mackenzie—Larkin (41)
22. Lake George, Warren Co., New York—Juday (36)
24. Lake Nipigon, Ontario—Adamstone (1)
25. Lac La Ronge, Saskatchewan—Rawson (55)
29. Lake Amisk, Saskatchewan—Rawson (55)
38. Wollaston Lake, Saskatchewan—Rawson (55)

##### *Mysis relicta* only

3. Lake Simcoe, Ontario—Rawson (51)
9. Waterworks of Duluth, Minnesota—Tattersall (71)
12. Fayetteville Green Lake, Onondaga Co., New York—Eggleton (17)
13. Trout Lake, Vilas Co., Wisconsin—Juday and Birge (37)
14. Lake Leelanau, Leelanau Co., Michigan—Holmquist (32)
17. Lake Nipissing, Ontario—Langford (38)
23. Churchill Lake, Saskatchewan—Rawson (54)
26. George Lake, Manitoba—Stewart-Hay (70)
28. Lesser Slave Lake, Alberta—R.B. Miller, *in* Larkin (40)
33. Echo Lake, Saskatchewan—Rawson (personal communication)
34. Nuwuk Pond, Point Barrow, Alaska—Mohr (45), Holmquist (32)
35. West Hawk Lake, Manitoba—Stewart-Hay (70)
36. Canoe Lake, Saskatchewan—Rawson (personal communication)

39. Cree Lake, Saskatchewan—Rawson (55); also lake expansions lower down on the Churchill River—Rawson (personal communication)
41. Millsite Lake, Theresa Co., New York—Sibley (64, p. 132)
42. Upper Saranac Lake, Franklin Co., New York—Greene (26, p. 124)
43. Lake Champlain, New York—Greene (26, p. 122), Rimsky-Korsakov (57, p. 104)

*Pontoporeia affinis* only

27. Chamberlain Lake, Piscataquis Co., Maine—Norton (47)
30. Big Peter Pond, Saskatchewan—Rawson (54)
31. Lake Washington, Seattle, Washington—Scheffer and Robinson (59)
32. Shakespeare Island Lake, in Lake Nipigon, Ontario—Cronk (11)
37. Ile à la Crosse Lake, Saskatchewan—Rawson (54); also almost all lakes in Saskatchewan in the Churchill drainage where reasonable depth and cold water is found, except Cree Lake (Rawson, personal communication)
40. Lake Pagwachuan, Thunder Bay District, Ontario—Stewart-Hay (70)

MARINE AND BRACKISH-WATER OCCURRENCES

*Mysis relicta*

At 200-ft depth in the sea; on the beach; and in Elson Lagoon; Point Barrow, Alaska—Mohr (45), Holmquist (32)

*Mysis oculata*

Point Barrow, Alaska—Mohr (45), Holmquist (32)  
 Cape Lesburne, Alaska—Holmquist (32)  
 Bernard Harbour, Mackenzie—Schmitt (60), Holmquist (32)  
 Dobbin Bay, east Ellesmere Island—Holmquist (32)  
 Eastern Baffin Island (various localities)—Holmquist (32)  
 Greenland (numerous localities)—Stephensen (69), Holmquist (32)  
 Coast of Labrador (various localities)—Smith (66), Rathbun (50), Tattersall (71), Holmquist (32)

*Mysis litoralis* (all from Holmquist, 32)

Friday Harbour, Puget Sound, Washington—Banner (3), type locality  
 Coal Islands, Cape Lesburne, Alaska  
 Bering Strait, Alaska  
 Point Barrow, Alaska (in plankton, on the beach, and in Elson Lagoon)  
 Bernard Harbour, Mackenzie  
 Isaacson Island, Franklin  
 Bylot Island, Franklin  
 Baffin Island (various localities), Franklin  
 Goose Bay and Lake Melville, Labrador

*Pontoporeia affinis*

Point Collinson, Alaska—Shoemaker (63)  
 Ungava Bay, Quebec—Dunbar (14)  
 Gulf of St. Lawrence—Bousfield (6)

### The Origin of North American Relicts

The problem of the origin of North American relicts is inseparable from that of the origin of the corresponding relicts in Eurasia. It revolves around two questions: Was there a single place of origin of all fresh-water *M. relicta* and *P. affinis*, or have they been developed from a marine form in two or more places? In either event, when did the fresh-water forms arise?

#### Multiple-origin Hypothesis

When organisms resembling arctic marine forms were first found in the Baltic Sea and neighboring lakes, it was widely accepted that they represented animals of salt-water origin that had become adapted to fresh water during the glacial period. Their occurrence inland was at first ascribed to flooding of the Baltic littoral during a post-glacial phase of land depression, followed by withdrawal of the sea, and retention of the animals in depressions that became lakes. Thus *Mysis relicta* was considered a fresh-water form of the marine *M. oculata*, while *Pontoporeia affinis* was derived from the marine *P. femorata*. When, a little later, relict species of similar or identical appearance turned up in North America, it was assumed that similar causes had produced similar effects—that an independent evolution, or perhaps just a phenotypic change, had modified the marine species similarly in both places.

Certain recent discoveries also support such a view. (1) Lomakina (42, p. 120) says that *Pontoporeia femorata* from brackish waters of the Sea of Japan, Siberia, the Caspian Sea, and the Baltic exhibit various degrees of reduction of the bifurcate process and great individual variability; extreme forms are almost identical with the totally reduced process of *affinis*. (2) The discovery of *P. affinis* in Kamchatka by Derzhavin (13), and in the State of Washington by Scheffer and Robinson (59), strongly suggests independent formation of the fresh-water form from a salt-tolerant ancestor. (3) Occurrence of *Mysis relicta* in ponds near the sea, remote from other fresh-water localities, suggests evolution in situ. Mohr (45) collected *Mysis* at Point Barrow, Alaska, in salt water, in a coastal lagoon, and in a fresh-water pond nearby. He concluded that "wherever stocks of *M. oculata* are confined to weakly brackish or fresh water, breeding individuals develop which, particularly in the character of the second antenna and the telson, resemble juvenile *M. oculata* but are in current practice assigned to the separate species *M. relicta*". Although Mohr's specimens included what Holmquist (32) now assigns to *litoralis*, his conclusions remain plausible. (4) The *Mysis relicta* of lakes of west Britain and Ireland are isolated from other European finds, the nearest being in southwestern Norway, and are most easily accounted for by independent evolution from a salt-tolerant marine form; though Segerstråle believes an all-fresh-water proglacial route from the Baltic region to have been possible.

#### Single-origin Hypothesis

The hypothesis of a single origin for fresh-water-adapted relicts received its first impetus when it was found that both *Pontoporeia affinis* and *P.*

*femorata* live together in the Baltic Sea without intergradation, or at any rate without complete intergradation. Ekman (19) observed that the farther north one goes in the Baltic, the less common is *femorata* and the more common is *affinis* until, at a salinity of less than 6 parts per thousand, *femorata* disappears altogether. Because of the absence of complete intergradation, Ekman decided that *P. affinis* in fresh water could not be a relict version of *femorata*, but rather that *affinis* itself was originally a brackish-water species that was or became adapted to fresh water also. This view was strengthened when *P. affinis* was collected in coastal arctic waters of Siberia, and lately from three American coastal localities. Similarly, Holmquist (32) now regards some of the supposed intergrades between *M. oculata* and *M. relictata* as a distinct species *M. litoralis*. Often more than one of these three species occurs in the same collection, and on one occasion she found all three. Furthermore *M. relictata*, like *P. affinis*, has been taken in arctic brackish waters, including some of fairly high salinity (at Point Barrow).

#### *Hypothesis of Pleistocene Origin*

Among those who favor a single origin for relict species there is sharp divergence of opinion as to the probable place and time where it occurred. According to Gurjanova (27), fresh-water *Pontoporeia affinis* probably originated during the Pleistocene in the part of Siberia between the Kara and Chukchi seas. Segerstråle (62), following P. L. Pirozhnikov, pictures the northern part of this region as a shallow sea in preglacial or interglacial times, which during the greatest (Riss/Illinoian) glaciation was invaded by ice sheets from highlands on both sides. The sheets coalesced, trapping a shallow sea to the southward that soon freshened to become a large proglacial lake. Lying approximately between the present Ob and Yenisei Rivers, this body of water discharged southward into the Aral, Caspian, and eventually the Black Sea. This may have been the place and time when all the marine relicts acquired their tolerance of fresh water. At any rate, Segerstråle considers that it was at this time that the central Asiatic amphipod *Pallasea quadrispinosa* reached northern Europe by a direct fresh-water connection. This species has no tolerance of salt water: its relatives live in Lake Baikal; hence it must have travelled west by an all-fresh-water route crossing some pass in the Ural Mountains. Other relicts might very well have made the same journey at the same time, but only the warmth-tolerant shallow-water *Pallasea* would be likely to survive a whole interglacial period in fresh water. For the other relicts, all cold-loving and to some extent salt-tolerant, Segerstråle believes that a postglacial invasion (or reinvasion) of the Baltic region from the White Sea via an Onega Ice Lake is the more likely immediate source.

In the east, Segerstråle (62, p. 105) favors dispersal of relicts by a fresh-water route: he believes that the Siberian ice-dammed lake, mentioned above, was connected with other proglacial waters far to the east, perhaps as far as North America, but gives no details. However, *Pallasea* has not found its way to North America, and it also failed to reach the British Isles. It

would seem more likely that *P. affinis* and *M. relicta* reached North America from Siberia in brackish water along the coast of the polar sea, possibly at a time when Bering Strait was closed and hence coastal waters in that part of the Arctic were presumably fresher than they are today.

#### *Hypothesis of Tertiary Origin*

The views of Holmquist (32) are quite different. She supposes that one or more species of *Mysis* inhabited a sea which covered most of European Russia during Eocene and Oligocene times. The modern species may have become differentiated then, or during the Miocene when this body of water became divided into a southern and a northern section as the continent rose. She is dubious whether the species *relicta* has or ever had a wide distribution in salt or brackish water, and seems to imply that it evolved as a fresh-water inhabitant of lakes left behind by the receding north-Russian Miocene sea.

Nothing definite is suggested by Holmquist concerning the time or method by which *M. relicta* reached America or western Europe, but evidently the Upsalan Högbom's proglacial lake hypothesis is not in highest favor at Lund. From its north European center of origin *M. relicta* may, says Holmquist, have moved westward and eastward by the aid of "dilution of water", by "transport within excavations of the ice" of drifting floes, by "various transgressions and submersions", and hence "not necessarily...by damming up of ice".

Holmquist feels that the morphological and cytological differences between the species support a middle-Tertiary or earlier origin of *relicta*, because they are too great to be consistent with its differentiation from *oculata* or *litoralis* subsequent to, or even during, the glacial age. Only by polyploidy might this occur, but *relicta* has approximately the same number of chromosomes as its relatives. After discussing the possible meanings of "relict", she concludes that *M. relicta* is not a relict in any sense of the word, and that all the present species of *Mysis* had evolved long before the Pleistocene.

Concerning the above, the question of probable rates of evolution, in the absence of a fossil record, is one of the most debatable matters in biology. There are some well-authenticated examples of speciation that *must* have occurred since glacial or pluvial times: for example, various cyprinodont fishes of the North American deserts, or the endemic cyprinids of the Phillipine Lake Lanao, which had its origin with a lava flow a bare 5000 years ago. The differences between the *Mysis* species, as set forth in Holmquist's Table I, seem anything but extreme, and her cytological studies also do not suggest major differentiation, though they may support the distinctness of the contemporary species.

For *Pontoporeia*, all recent authors regard *affinis* as distinct from *femorata*, in spite of the variability of the latter in brackish water. Within *affinis*, Segerstråle (62) recognizes a subspecies *microphthalma* Sars from the Caspian Sea, but rejects the subspecies *gurjanovae* Birula, described from arctic estuarine waters, as equivalent to typical *affinis* from the Baltic. He "doubts whether characters of the form living in lakes...are sufficiently pronounced

to justify subspecific status" and, making a taxonomic distinction not usually recognized in America, proposes that it be regarded as "only a variety of the main form", which he names var. *lacustris* (62, p. 35). In North America several specific or subspecific names have been proposed, which are not recognized by Lomakina (42) or Segerstråle (61, 62). The most recent systematist to mention the matter suggests, briefly, that: "evidence to date could be interpreted to provide grounds for subspecific recognition of all North American material (as *P. a. hoyi* Smith), and that within this complex are one or more varieties of comparatively recent evolution. The rather large, morphologically and ecologically distinct form recorded under the name *Pontoporeia affinis* from littoral salt waters of Ungava Bay and the St. Lawrence estuary may prove specifically separable from Lindström's form" (Bousfield (7)).

### Conclusion

Whatever the final decision of taxonomists, it seems safe to conclude that there is a close similarity between the Eurasian and North American representatives of *P. affinis* and of *M. relicta*. Evidently, then, if the fresh-water forms originated independently on the two continents it must have been very recently; whereas if they originated only once, then the migration from the Old World to the New must also have been recent (glacial or post-glacial time). No hypothesis involving two *ancient* origins of the fresh-water-adapted species can be entertained, certainly not at the time of the last continental submergence of North America in the Upper Cretaceous.

For our purpose it is not necessary to come to any firm conclusion concerning the place or places of origin of the two fresh-water relicts. Specimens identical with or extremely close to fresh-water specimens occur to-day in arctic marine or brackish waters, though American records are few. Thus they may have existed in their present form in Canadian arctic waters in preglacial or interglacial times in sufficient numbers to provide the nucleus of the American fresh-water stocks. Their present scarcity in, or absence from, the central Canadian Arctic might be laid to the more severe marine climate there (as compared with Alaska and the eastern Arctic), or to competition from recently evolved species better adapted to those conditions. However it seems too early to exclude completely the old idea that *P. affinis* and *M. relicta* evolved rather recently from circumpolar marine precursors (either the modern species or a Pleistocene ancestor), and did so independently on more than one occasion. Or there might have been a single origin for one species and multiple origins for the other.

### Environmental Biology

A discussion of the distribution of *Mysis* and *Pontoporeia* in North American lakes must be prefaced by a brief consideration of their environmental requirements. Both species are intolerant of certain conditions of temperature, oxygen, and light that are common in some lakes.

*Mysis*

The distribution of *Mysis relicta* in fresh waters of North America is apparently limited by temperature and oxygen conditions. Although Thiennemann (72) set 4 cc per liter as the lowest concentration that German *relicta* can tolerate, Juday and Birge (37) found it in water with an oxygen content of less than 1 cc/l. in Trout Lake and Green Lake, Wisconsin. Pennak (49) reports that 40–50% oxygen saturation is the usual lower limit, but some are found in water as low as 20% saturated. Abundance of oxygen may also be undesirable. Holmquist (32) found by experiment that mortality of *Mysis relicta* followed within a few days when the animals were subjected to a "super-saturation" of oxygen obtained by bubbling pure oxygen through the water of an aquarium. The exact level of oxygen attained is not stated.

Samter and Weltner (58) and Pennak (49) found the upper temperature limit for *M. relicta* to be 14°C, but Larkin (41) kept the animals successfully at 16–18° provided they were in darkness. Dr. D. S. Rawson (personal communication) has found *Mysis* at 20° C in great numbers at night in Lac La Ronge, Saskatchewan.

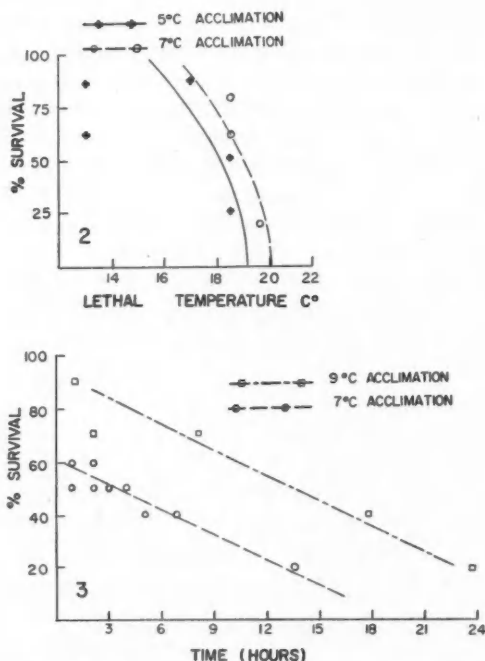


FIG. 2. Percentage survival of *Mysis relicta* as related to acclimation temperature. (From unpublished data of Dr. P. A. Larkin.)

FIG. 3. Percentage survival of *Mysis relicta*, from acclimation at 7° and 9° C, when subjected to a temperature of 19.5° C. (From unpublished data of Dr. P. A. Larkin.)

Dr. P. A. Larkin made some lethal temperature tests on *M. relicta* collected in September, 1950, from the Waterton Lakes, Alberta, whose results he has asked me to present here. The animals were acclimatized at 5°, 7°, 9°, 10°, and 14° C. Upper thermal limits varied from 18° to 22°, depending on the acclimation temperature. Figure 2 shows a comparison of the upper thermal limits for 5° and 7° acclimation, and Fig. 3 compares specimens acclimated to 7° and 9°, which were plunged into a beaker of water at 19.5° C, the time to death being noted. The rise in lethal temperature with increase in acclimation temperature is evident. Holmquist (32) observed a 50% mortality in 28 days when temperature was increased gradually from 13.5° to 18° C. A further 30 days at 18° killed all the remaining animals.

*Mysis relicta* has not been collected from running waters, although Southern and Gardiner (67, p. 88) found it in a deep slow portion of the River Shannon a mile below Lough Derg. It has never been observed in the field to swim against any considerable current, and this feature of its biology has been emphasized by Holmquist (31) and many earlier workers as an important factor limiting its distribution. Holmquist (32) found experimentally that the animals would turn against a gentle current and take refuge in bottom materials, but stronger currents washed them away. *M. relicta* does undertake vertical diurnal movements in lakes (Larkin, (41), Pennak (49)).

Juday and Birge (37) and a few others have observed negative phototropism in *M. relicta*, and Southern and Gardiner (67) found immature specimens to be less sensitive to light than adults. Larkin (41) found *Mysis* in shallow turbid waters of Great Slave Lake; he suggested that possibly light as well as temperature affects the vertical distribution of this species. Holmquist (32) postulates that supersaturation of oxygen in the epilimnion of lakes may account for their frequent absence from surface waters.

Samter and Weltner (58) observed breeding of *Mysis relicta* at 7° C or lower, in the winter. Ekman (18-20), however, observed summer breeding in Lake Vättern, and Thienemann (72) found that where summer reproduction occurs it is always in an oligotrophic lake.

Holmquist (32) transferred *M. relicta* directly from fresh water to a salinity of 7 parts per thousand without serious mortality.

From these observations it is clear that *Mysis relicta* can tolerate a fairly wide range of lake conditions, but it appears to flourish best where oxygen is abundant and temperatures are low.

#### *Pontoporeia*

Juday and Birge (37) noted the presence of *Pontoporeia affinis* in Green Lake and Trout Lake, Wisconsin, in places where oxygen was less than 1 cc/l., while Pennak (49) reports it in water of less than 7% saturation. Although usually a cold-water species, Samter and Weltner (58) found *P. affinis* in fairly warm lakes. Gurjanova (27) did not find *P. affinis gurjanovae* (= *P. a. affinis*) in waters of salinity greater than 7 parts per thousand in the estuaries of north Siberian rivers.

According to Ekman (18-20), Samter and Weltner (58), and Juday and Birge (37), *Pontoporeia affinis* does not have a summer breeding season. The salt-water form can be distinguished physiologically from the fresh-water form by the length of time to reproduction—the latter will breed after 1 year's growth, whereas the marine form requires 2 years. Larkin (41), however, observed summer breeding in Great Slave Lake; like Samter and Weltner, he found that breeding does not occur at temperatures greater than 7° C, and individuals which breed in summer below the 30-meter level give birth to their young in the early winter months.

Thus *Pontoporeia affinis* appears to be mainly a cold-water species, tolerant of low oxygen concentrations but requiring low temperatures. Rawson (51) postulated that *P. affinis* once inhabited Lake Simcoe, Ontario, and that its absence to-day is a result of the gradual warming of the lake since glacial times.

### Distribution of Relicts in the Fresh Waters of North America

Since *Mysis relicta* and *Pontoporeia affinis* are found in lakes only in glaciated areas, European biologists developed two principal hypotheses for their distribution in the Baltic and Scandinavian region (see the discussion by Juday and Birge (37)). The theory of Samter and Weltner (58) is that relicts originally occupied ocean basins; subsequent elevation of the land brought these basins above sea level, after which inflow gradually freshened the water. The second view is that during the last glacial epoch the future glacial relicts occurred in salt or brackish water at the front of the ice centers in places where the glacier advanced over shallow seas or estuaries, and were "pushed onto land" in the proglacial lakes which always exist at various points along an ice front when it is advancing up a land gradient (or when it is retreating down one). This is the so-called "sluicing-up theory" of Högbohm (29), here called the "proglacial lake theory".

The distribution of glacial relicts in North America has not been adequately explained to date, partly because of the inadequacy of geological information. Marsh (43) speculated on the origin of relict Crustacea in Green Lake, Wisconsin, a lake of glacial origin with a huge drift dam at the west end. In general his views resembled the Samter and Weltner theory, but he believed the Green Lake occurrences could not be explained in that way because the lake was never a part of the Great Lakes. He suggested that waterfowl must have brought the relict species to Green Lake. Applied generally, waterfowl transportation would be very unlikely to have produced the present North American (or other) distribution of relicts, because (1) *Mysis* and *Pontoporeia* are usually deep-water species unlikely to be transported by birds, and (2) these relicts are not found in the highland lakes of British Columbia or Quebec, where birds could presumably have taken them if that mode of transport were feasible over a distance. Neither of these species has developed resistant eggs or resting stages which would facilitate overland transport (Holmquist (32)).

Gurney (28) explained the presence of the relicts *Limnocalanus macrurus* and *Mysis relicta* in the Great Lakes as a combination of a late postglacial marine inundation which reached Lake Champlain and Lake Ontario, and an upstream migration to the upper lakes by way of the Ottawa valley and Lake Nipissing, which at one time drained Lake Huron. He indicated that this required an active upstream migration through some formidable rapids, which seems far beyond the capacity of either species.

Ortman (48), Högbom (29), Ekman (21), Thienemann (73), and Segerstråle (62) favor the Högbom proglacial lake mechanism to account for relicts in the Great Lakes and other lakes of North America, but give no details.

Larkin (40) summarized the North American records of *Pontoporeia* and *Mysis* and discussed the various theories which might account for their distribution. He noted the close association between the areas of maximum glaciation and the southern limits of *Mysis* and *Pontoporeia*, but believed that the proglacial lake theory was inadequate. The presence of both species in the Waterton Lakes, over 1500 miles inland and at an elevation of more than 4200 ft, seemed beyond explanation in the light of theories of glaciation then current. The then-accepted view placed the Keewatin Ice Centre far inland (Fig. 1), so that there would be no possibility of the advancing glacier traversing a brackish or marine source of the organisms en route to these lakes, although this would have been possible in the case of the Great Lakes, if Keewatin ice had moved southeasterly through southern Hudson Bay before it crossed the divide in northern Ontario. Larkin concluded by suggesting a preglacial marine invasion of the Samter and Weltner type, though the latest geological evidence of mid-continental seas dates back to the Upper Cretaceous.

A recent theory of the glacial history of North America makes it possible to reconcile these contradictions and permits a straightforward explanation of the distribution of our glacial relicts. Instead of three major ice centers in North America, Flint (23; 24) postulates only two: the Laurentide Ice Sheet of the eastern and middle parts of the continent, and the Cordilleran Ice Sheet of the western mountain ranges. According to Flint, the Laurentide sheet originated in (1) the mountains of Baffin and Ellesmere Islands, (2) the mountains of coastal Labrador, and (3) the highlands of eastern Quebec (Fig. 1). The formerly accepted Keewatin mid-continental ice center did not exist, in his opinion. Explorations in the little-known country east and west of Hudson Bay had revealed the existence of striations pointing more or less radially outward from several ill-defined areas in these two regions, and for some time it was generally believed that these areas were the "centers" from which the ice spread out—the Labradorian and Keewatin ice centers. However, subsequent investigations showed that the striations from which these "centers" were inferred must have been made during a very late phase of the glaciation and have little significance.

Flint had and has little to offer as evidence for the exact location of his ice centers, but because the Labradorian ice center of the Laurentide Ice Sheet is related to a highland situated so as to receive heavy snowfall, he

believed it to be the area of a separate ice sheet which was formed by coalescence with glaciers flowing outward from other highlands in northeastern North America. According to this hypothesis, the early Quebec-Labrador ice sheet and the Baffin-Ellesmere ice sheet coalesced over Hudson Strait, thereby creating the Laurentide Ice Sheet proper (Flint (24)). Meanwhile, the ice on the western slope of Baffin Island pushed down to form piedmont glaciers on the lowland now occupied by Foxe Basin and northern Hudson Bay (Flint (23)). Thus any potential relicts in the seas of the Canadian archipelago would be picked up in the proglacial lakes formed in front of the ice sheet as it spread from Baffin and Ellesmere Islands southward. As the ice was gradually pushing its way uphill and as the front was almost certainly uneven, water would be trapped constantly some place or other at the front of the ice margin. Thus adaptable members of the marine or brackish fauna—the future relicts among them—were gradually pushed across North America in a radial fashion from the ice center, in lakes well supplied with incoming water. Upon reaching the greatest extent of glaciation, the relict fauna inhabited lakes formed by the water dammed between the ice edge and the hills or mountains in front of the ice. When recession began, various excavated basins, valleys blocked by moraines, intermorainal depressions, etc. provided a tremendous variety of lacustrine habitats in which the relicts lived as the ice-margin slowly and irregularly retreated across the continent (Figs. 4, 5). The “plunge-basin” lake near Oneida Lake, N. Y., excavated by a cataract from the ice front, is one of the more spectacular types, and one which contains *Mysis relicta* (Eggleton (17)).

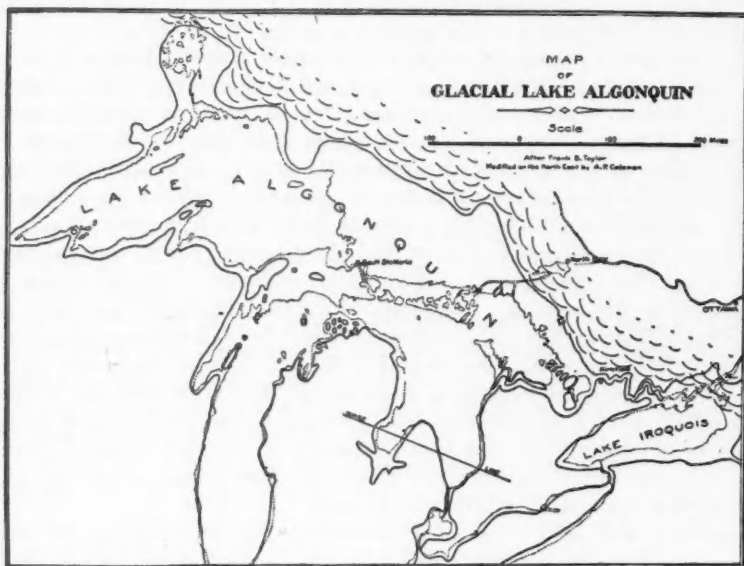


FIG. 4. Map of Lake Algonquin. (From Coleman (10).)

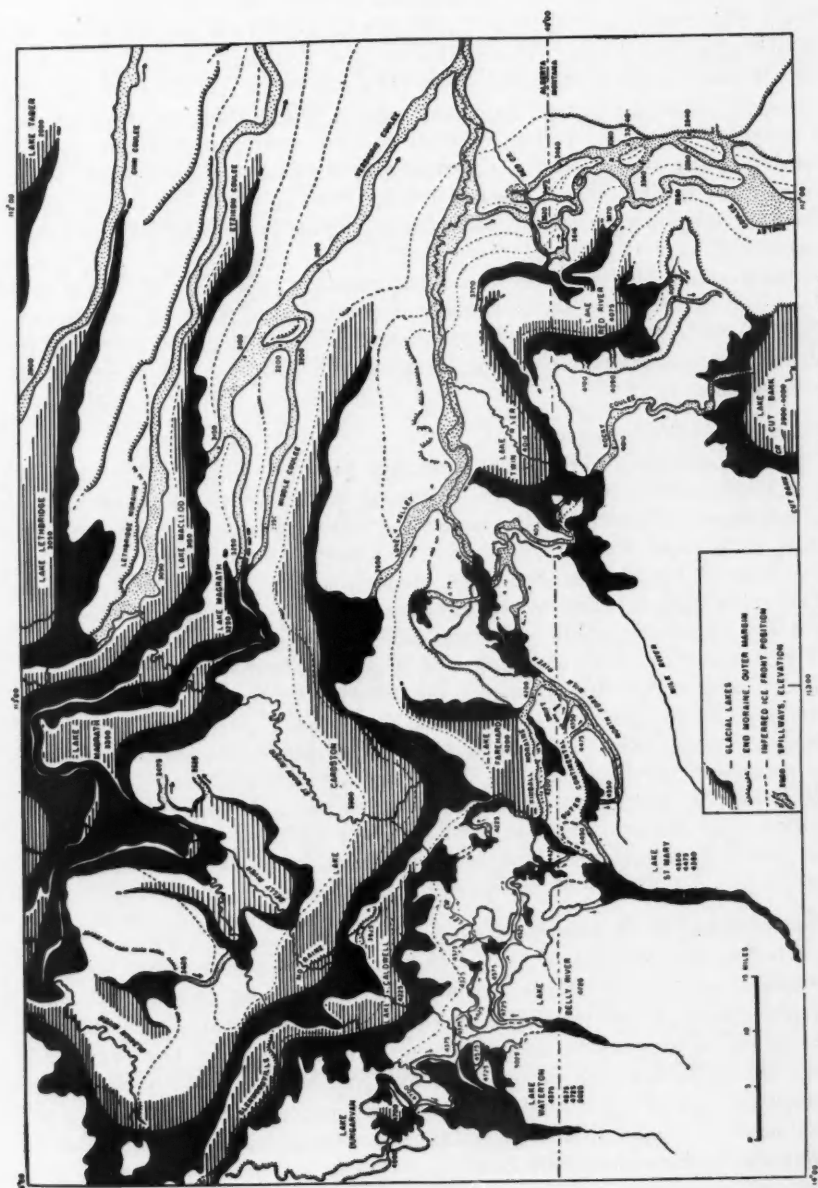


FIG. 5. Glacial geology of the Waterton region. (From Horberg (33).)

The fate of the fauna in such proglacial lakes depended principally on whether there were any deep depressions in the basin. Great Bear Lake, Great Slave Lake, and the Great Lakes are examples of the bigger depressions in the proglacial lakes. These lakes occupy preglacial valleys converted into basins by glacial corrasion (Flint (23), Flint *et al.* (25)). Cameron (8) concluded that the run-off from the eastern Rocky Mountains would be impounded by the retreating ice front to form lakes which would expand laterally along the ice sheet. Coleman (10) describes the formation of Lake Algonquin by the various lobes of the ice sheet gradually shrinking, with the ice north of the present day lakes Huron and Superior forming its northern coast (Fig. 4).

Examination of Fig. 1 indicates that almost all records of *M. relicta* and *P. affinis* occur where an ice-dammed lake was present, or at least where the modern lake lies on glaciated terrain. The latter are also probably mostly descendants of Pleistocene lakes for which there is lack of geological information.

#### *Relicts in the Waterton Lakes*

The proglacial lake theory can account for the presence of *Pontoporeia* and *Mysis* in the Waterton Lakes. Although rather remote from other known occurrences, distance alone presents no greater obstacle in North America than in Europe; Högbom originally (29) proposed his theory to suggest an origin of the Caspian Sea relict fauna from the Arctic Ocean, 1200 to 1500 miles distant. The Waterton Lakes are about the same distance from a source of marine relicts. Horberg (33) has mapped an ice-dammed lake in the Waterton area, including and extending beyond the modern lake basins, which was 5025 ft above the present sea level. Figure 5, from Horberg, shows a number of additional, but extinct, proglacial lakes in the general region (lakes Caldwell, Magrath, MacLeod, Lethbridge, and Taber, in succession northeastward). Two other ice-dammed lakes (lakes Belly River and St. Mary (Fig. 5)) filled valleys of the east slope of the Rockies southeast of Waterton Lakes; Lake St. Mary survives to-day, reduced in size but still quite deep, and it is a good guess that *Pontoporeia* and *Mysis* will be found there as soon as someone takes a look.

#### *Pontoporeia affinis* in Lake Washington

Scheffer and Robinson's (59) discovery of *Pontoporeia affinis* in Lake Washington remains the only record of this species west of the continental divide. Dr. W. T. Edmondson of the University of Washington has confirmed the identification (personal communication). Were it not that Derzhavin (13) reported the species from various lakes and rivers of Kamchatka, some wholly fortuitous type of dispersal for this lake might perhaps be considered. As it is, it must be accepted that in glacial times or during deglaciation *Pontoporeia* extended far down the Pacific coast in the colder and possibly more brackish water of those times, and has since probably disappeared from the sea because of higher temperatures or salinities.

Figure 6 shows the glacial geology of the Puget Sound region. Along the coast of British Columbia the Cordilleran Ice Sheet pushed its way into the sea. Vancouver Island had its own ice cap which met the Cordilleran one in the Strait of Georgia, the combined mass moving northwestward and southward and southwestward, out of the two ends of the Strait (Mathews

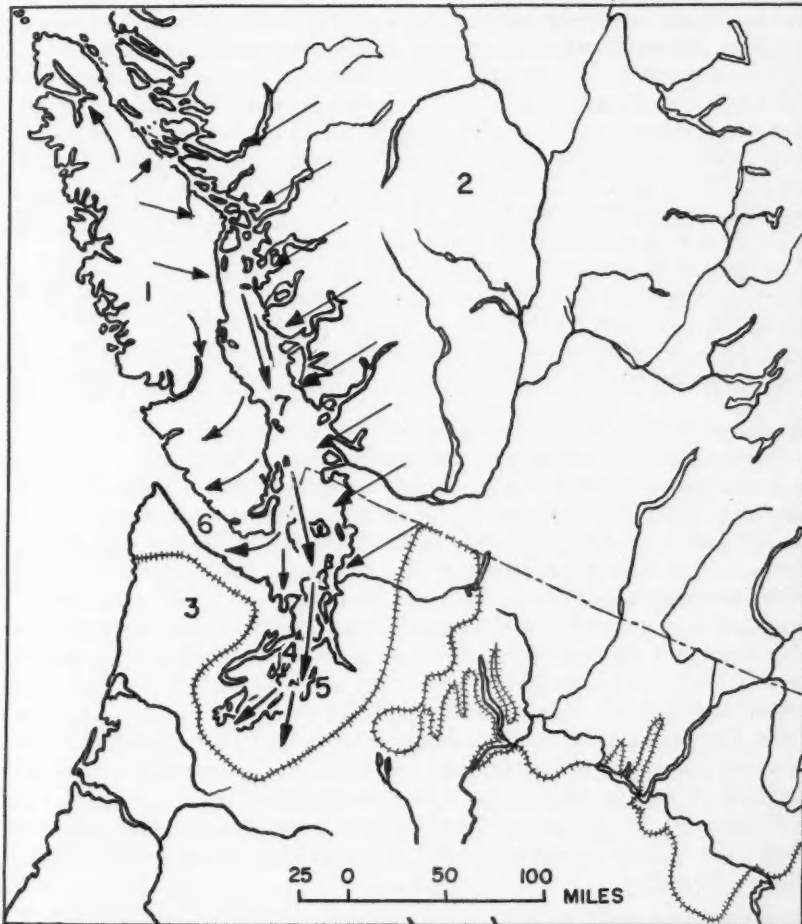


FIG. 6. Glacial map of Vancouver Island, Puget Sound, and the mainland, showing directions of ice flow. (From Flint *et al.* (25) and Mathews (44).)

- |  |                           |
|--|---------------------------|
| 1. Vancouver Island Ice Cap                            | 4. Puget Sound            |
| 2. British Columbia Ice Cap<br>(Cordilleran Ice Sheet) | 5. Lake Washington        |
| 3. Olympic Ice Cap                                     | 6. Strait of Juan de Fuca |
|  | 7. Strait of Georgia      |

Arrows indicate direction of ice flow.

The double-hatched line marks the southern limit of movement of ice from the Cordilleran Ice Sheet (i.e., excluding local ice from the Olympic and Cascade Mountains).

(44), Flint (24)). The southern edge of the coalesced ice mass pushed down into the Puget Sound region as a huge tongue. At this time, any potential relict species in the Strait of Georgia or Puget Sound would be pushed ahead in a proglacial lake covering the lowlands of northwestern Washington. The Olympic Mountains ice cap did not stand in the way, because this cap was receding while the cordilleran was still advancing in this area (personal communication from Professor W. R. Danner, University of British Columbia). At its greatest extent the Puget Sound ice tongue crossed the height of land southward toward the Columbia River; however, smaller lakes were presumably dammed back by the ice in side valleys of the Cascade or Olympic Mountains, some of which would harbor the relicts until retreat of the tongue re-established the proglacial expanse, one of whose remnants is Lake Washington. If this picture is correct, *Pontoporeia* will probably be found in other deep lakes of the Puget Sound lowland, such as Lake Samamish, and even in Crescent Lake bordering the Strait of Juan de Fuca.

In spite of fairly extensive limnological surveys, there are no records of *Pontoporeia* in fresh waters of the British Columbia mainland or Vancouver Island (Ricker (56), Northcote and Larkin (46)). However, there seems to be no part of the British Columbia coast where the topography would allow proglacial lakes to form: usually the ice pushed directly down to the sea.

#### *Absence of Relicts from Eastern Highlands*

Just as relicts are absent from the cordillera proper, so they do not appear in some apparently suitable parts of eastern Canada, a fact noted by Bousfield (*in* Stewart-Hay (70), discussion). As mentioned above, one of the ice centers for the Laurentide Ice Sheet was in the highlands of Quebec—specifically, those north of the St. Lawrence River and Gulf including the Parc des Laurentides, the Shickshock Mountains, and Newfoundland (Flint (24), Fig. 1). Therefore the ice could not have passed through any marine source to place glacial relicts in most of Quebec, but it did pass through the salty St. Lawrence estuary and Gulf to populate lakes on the south side of the St. Lawrence: known localities are Millsite Lake, Upper Saranac Lake, Lake George, and Lake Champlain in New York, and Chamberlain Lake in Maine. The last-named does not now drain into the Gulf of St. Lawrence, but the divide between its drainage and the St. Lawrence is not very high.

Presumably for the same reason, *Mysis relicta* has not been taken on Baffin Island, another center of the Laurentide Sheet—a fact which puzzled Holmquist (32).

#### *Mysis relicta at Point Barrow*

At Point Barrow, Alaska, *Mysis relicta* is reported from the sea, from a coastal lagoon, and from a fresh-water pond, Nuwuk Pond; the records have been checked by Holmquist (32) and numerous specimens were available from all three localities, so they must be considered completely authentic. This part of Alaska was not glaciated, so the fresh-water occurrence cannot have anything to do with proglacial lakes. Possibly this is one place where *Mysis* has been "stranded" by elevation of a rising coast line in very recent

times, geologically. Transportation by waterfowl may be another possibility: Summerhayes and Elton (68) have reported Arctic terns carrying *Mysis* for short distances on Spitsbergen, and as a matter of fact Mohr (45) reports on the feeding activities of several water birds at Nuwuk Pond, which is close to the coast. Transportation by the wind in spray can also be considered, as Mohr observed that salt spray reached the pond during a severe autumn storm. Whatever the mechanism, it is unnecessary to link up seashore pond records with the same process as produced the inland continental distribution of the relicts.

### Conclusion

The distribution of *Mysis* and *Pontoporeia* in continental North America can be satisfactorily explained on the basis of proglacial transport. The preglacial marine invasion suggested by Larkin and others has no geological support if it is placed in Tertiary time, while a Cretaceous (or even considerably later) invasion and subsequent isolation is inconsistent with the observed close resemblance between American and Eurasian relicts. In any event a pre-Pleistocene marine invasion is not necessary to explain relict distribution if Flint's recent glacial hypothesis is acceptable. Indeed, the distribution of the relicts is excellent evidence supporting the hypothesis of only two ice sheets.

Larkin (41), Rawson (52), and others have shown that *M. relicta* and *P. affinis* are a valuable source of food for lake trout, *Salvelinus namaycush*, and other fishes. As a result, they have been planted in Kootenay Lake, B. C. (P. A. Larkin, personal communication). Dr. Larkin has also been advised (by Dr. R. W. Pennak) of their introduction into high lakes of Colorado, and (by Dr. J. H. Wales) of a proposal to introduce *Mysis* into Castle Lake, California, as well as (by Dr. R. Vincent) of plans to place them in certain lakes of New York. Thus future records of relicts in these regions must be checked against artificial introductions.

### Summary

1. The location of glacial relicts in lakes which are remnants of Pleistocene ice-dammed lakes, the environmental physiology of the relicts, and the position of the ice centers postulated by Flint are together consistent with the distribution of North American glacial relicts in the manner proposed (for Europe) by Högbom, which pictures the spread of the relicts from Arctic marine or brackish habitats in a series of proglacial lakes pushed in front of the ice sheet as it advanced, and following it back north as it retreated.

2. Only Flint's hypothesis of *two* major ice sheets is completely consistent with relict distribution in the Högbom manner. These two are the Cordilleran Ice Sheet, and over the rest of Canada a single Laurentide Ice Sheet which advanced west and south from the highlands of Ellesmere Island, Baffin Island, and eastern Quebec and Labrador. The alternative hypothesis, which included a Keewatin Ice Centre, could not have moved relicts to the Waterton Lakes, Alberta.

3. Glacial relicts of North America are believed to have originated in marine waters of the Canadian Arctic Archipelago, and were pushed southward ahead of the advancing Laurentide Ice Sheet. The Gulf of St. Lawrence is a possible secondary source of eastern relicts, though a connection westward is not excluded.

4. Parent stocks of *Pontoporeia affinis* and *Mysis relicta* in North America may stem originally from the evolution of these species, or the evolution of their fresh-water tolerance, in the great central Siberian Pleistocene ice-dammed lake, followed by migration eastward in brackish waters along the Arctic coast. Alternatively, separate Eurasian and American evolution of these species from close marine relatives cannot yet be excluded, particularly in the case of *Mysis*.

5. Migration of our relict species from the Siberian lake to North America by an all-fresh-water route (as suggested by Segerstråle) does not appear probable, because of the absence of the amphipod *Pallasea quadrispinosa* in North America.

6. The upper thermal limits for *Mysis relicta* are from 18° to 22° C.

7. The Waterton Lakes lie close to the southwestern border of the Laurentide Ice Sheet; they lie in the basin of a former ice-dammed lake, one of the last of a series of proglacial lakes in which *Mysis* and *Pontoporeia* were pushed southwestward from their origin in arctic waters.

8. The presence of relicts in lakes of eastern New York and in Chamberlain Lake, Maine, is consistent with known or possible ice-dammed lakes in that area. Proglacial lakes in this area could not develop north of the St. Lawrence valley because the ice border there was moving downhill during glaciation and retreating uphill during deglaciation; and no relicts have been reported north of the St. Lawrence.

9. Similarly *Pontoporeia* in Lake Washington may be related to a late glacial advance of a tongue of ice from the north into Puget Sound and southward, resulting in the development of a proglacial lake or lakes of which Lake Washington is one remnant. This stock originated independently of the main continental stock, from marine or brackish-water ancestors.

10. The "relicts" of Nuwuk Pond, Alaska, and any other arctic ponds near the sea where their occurrence may be verified, did not arrive there as a result of glacial transport but by some other mechanism: probably acclimatization in situ.

#### Acknowledgments

I wish to thank Dr. P. A. Larkin for suggesting this study, for making available unpublished experimental data, and for assistance in preparing the manuscript for publication. Dr. W. H. Mathews gave invaluable aid with the geological problems and checked the final manuscript. Dr. W. E. Ricker read the manuscript and assisted with translations from German and Russian. Mr. J. Van Tets helped with German translations. The maps and graphs are the result of painstaking work by Mr. R. D. Lyon. Dr. D. S. Rawson

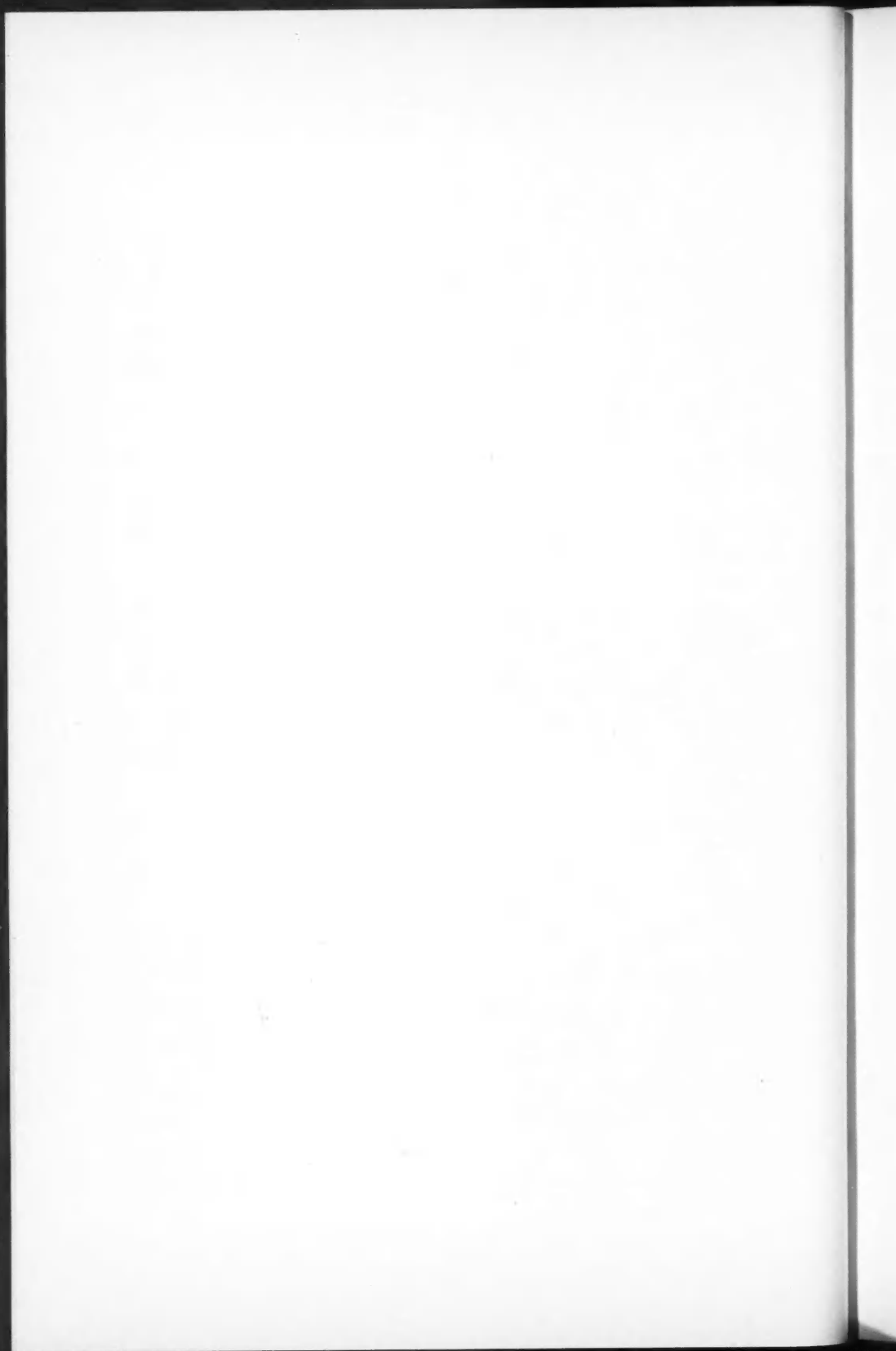
provided recent Saskatchewan distribution records, and critically examined the manuscript. Mr. R. Stewart-Hay provided recent Manitoba locality records, while Mr. B. Kooyman identified some obscure geographical locations. Dr. C. C. Lindsey generously rechecked the manuscript. Miss M. Jurelka and Miss L. Campbell Brown assisted by typing various drafts of the manuscript.

### References

1. ADAMSTONE, F. B. The distribution and economic importance of the bottom fauna of Lake Michigan, with an Appendix on the bottom fauna of Lake Ontario. Univ. Toronto Studies, Biol. Ser. No. 25; Publ. Ontario Fisheries Research Lab. No. 24, 33-100 (1924).
2. BAJKOV, A. Biological conditions of Manitoba lakes. Contrib. Can. Biol. and Fisheries, 12, 383-433 (1930).
3. BANNER, A. H. New records of Mysidacea and Euphausiacea from the Northeastern Pacific and adjacent areas. Pacific Science, 8(2), 125-139 (1954).
4. BIRGE, E. A. and JUDAY, C. A limnological study of the Finger Lakes of New York. Bull. U. S. Bureau Fisheries, 33, 525-609 (1914).
5. BIRGE, E. A. and JUDAY, C. Further limnological observations of the Finger Lakes of New York. Bull. U. S. Bur. Fisheries, 37, 209-252 (1921).
6. BOUSFIELD, E. L. Studies on the shore fauna of the St. Lawrence estuary and Gaspé coast. Bull. Natl. Museum Can. 136, 95-101 (1955).
7. BOUSFIELD, E. L. Fresh-water amphipod crustaceans of glaciated North America. Can. Field-Naturalist, 72(2), 55-113 (1958).
8. CAMERON, P. E. Post glacial lakes in the Mackenzie River basin, North West Territories, Canada. J. Geol. 30(5), 337-353 (1922).
9. CARPENTER, K. E. Life in inland waters. Sedgwick and Jackson, Ltd., London. 1928.
10. COLEMAN, A. P. Glacial and postglacial lakes in Ontario. Univ. Toronto Studies Biol. Ser. No. 21; Publ. Ontario Fisheries Research Lab. No. 10, 1-76 (1922).
11. CRONK, M. W. The bottom fauna of Shakespeare Island Lake, Ontario. Univ. Toronto Studies Biol. Ser. No. 36; Publ. Ontario Fisheries Research Lab. No. 43, 29-66 (1932).
12. CUERRIER, J.-P. and SCHULTZ, F. H. Studies of lake trout and common whitefish in Waterton Lakes, Waterton Lakes National Park, Alberta. Can. Natl. Parks Branch, Wildlife Management Bull. 3(5), 1-41 (1957).
13. DERZHAVIN, A. N. [Malacostraca of the fresh waters of Kamchatka.] Russ. Gidrobiol. Zhur. 2, 180-194 (1923).
14. DUNBAR, M. J. The amphipod Crustacea of Ungava Bay, Canadian Eastern Arctic. J. Fisheries Research Board Can. 11(6), 700-798 (1954).
15. EATON, E. H. The Finger Lakes fish problem. Suppl. 17th Ann. Rept. N. Y. Conserv. Dept. 40-66 (1928).
16. EGGLETON, F. E. The deep water fauna of Lake Michigan. Papers Michigan Acad. Sci. for 1935, 21, 599-612 (1936).
17. EGGLETON, F. E. Limnology of a meromictic, interglacial, plunge-basin lake. Trans. Am. Microscop. Soc. 75(3), 334-378 (1956).
18. EKMAN, S. Studien über die marinen Relikte der nordeuropäischen Binnengewässer. IV. Welche ist die marine Stammform der Relikten *Pontoporeia affinis* Lindström? Intern. Rev. Hydrobiol. Hydrogr. 8, 321-331 (1918).
19. EKMAN, S. Studien über die marinen Relikte der nordeuropäischen Binnengewässer. VI. Die morphologischen Folgen des Reliktwerdens. Intern. Rev. Hydrobiol. Hydrogr. 8, 477-528 (1919).
20. EKMAN, S. Studien über die marinen Relikte der nordeuropäischen Binnengewässer. VII. Fortpflanzung und Lebenslauf der marin-glazialen Relikte und ihrer marinen Stammformen. Intern. Rev. Hydrobiol. Hydrogr. 8, 543-589 (1920).
21. EKMAN, S. Die südbaltischen marin-glazialen Relikte und die Stauseetheorie. Intern. Rev. Hydrobiol. Hydrogr. 24, 225-243 (1930).
22. EKMAN, S. Zoogeography of the sea. Sedgwick and Jackson, Ltd. London. 1953.
23. FLINT, R. F. Glacial geology and the Pleistocene epoch. John Wiley and Sons, Inc., New York. 1947.
24. FLINT, R. F. Glacial and Pleistocene geology. John Wiley and Sons, Inc., New York. 1957.
25. FLINT, R. F. et al. Glacial map of North America. Geol. Soc. of North Am. (1945).
26. GREENE, C. W. The smelts of Lake Champlain, with supplementary material from the Finger Lakes, the Saranac chain, and the Cold Spring Harbour Hatchery, Long Island, N. Y. Suppl. 19th Ann. Rept. N. Y. Conserv. Dept. 105-129 (1930).

27. GURJANOVA, E. F. [The amphipods of the seas of the USSR and adjacent waters (Amphipoda—Gammaridea).] Akad. Nauk SSSR, Opredeliteli po Faune SSSR, No. 41, 1–1029 (1951).
28. GURNEY, R. The crustacean plankton of the English Lake District. J. Linnean Soc. Zool. **35**, 411–447 (1923).
29. HÖGBOM, A. G. Über die arktischen Elemente in der aralokaspischen Fauna, ein tier-geographisches Problem. Bull. Geol. Inst. Univ. Upsala, **14**, 241–260 (1917).
30. HOLMQUIST, C. Über eventuelle intermediäre Formen zwischen *Mysis oculata* Fabr. und *Mysis relicta* Lovén. Lunds Univ. Årsskr. N. F. Avd. 2, **45**(10), 1–26 (1949).
31. HOLMQUIST, C. Die *Mysis relicta* aus dem Mittelmeergebiet (= *Diamysis bahirensis* (G. O. Sars) 1877 und *Paramysis helleri* (G. O. Sars) 1877). Zool. Anz. **154**, 277–288 (1955).
32. HOLMQUIST, C. Problems on marine-glacial relicts on account of investigations on the genus *Mysis*. Berlingska Boktryckeriet, Lund, 1959.
33. HORBERG, L. Rocky Mountain and continental Pleistocene deposits in the Waterton region, Alberta, Canada. Bull. Geol. Soc. Am. **65**, 1093–1150 (1954).
34. HUNTSMAN, A. G. The freshwater Malacostraca of Ontario. Contrib. Canadian Biol. **1911–14**(II), 145–163 (1915).
35. JOHANSEN, F. The Crustacea life of some Arctic lagoons, lakes and ponds. Rept. Canadian Arctic Exped. 1913–18, **7**(N), 1–31 (1922).
36. JUDAY, C. Limnological observations on Lake George. In A biological survey of Lake George. N. Y. State Conserv. Comm. 1922. pp. 37–51.
37. JUDAY, C. and BIRGE, E. A. *Pontoporeia* and *Mysis* in Wisconsin lakes. Ecology, **8**, 445–452 (1927).
38. LANGFORD, R. R. The food of the Lake Nipissing cisco, *Leucichthys artedi* (LeSueur), with special reference to the utilization of the limnetic Crustacea. Univ. Toronto Studies, Biol. Ser. No. 45; Publ. Ontario Fisheries Research Lab. No. 57, 149–183 (1938).
39. LANGLOIS, T. H. The western end of Lake Erie and its ecology. J. W. Edwards Co., Ann Arbor, Michigan. 1954.
40. LARKIN, P. A. *Pontoporeia* and *Mysis* in lakes of the Canadian Northwest. M. A. thesis, University of Saskatchewan, Saskatoon, Sask. 1946.
41. LARKIN, P. A. *Pontoporeia* and *Mysis* in Athabasca, Great Bear and Great Slave Lakes. Bull. Fisheries Research Board Can. **27**, 1–23 (1948).
42. LOMAKINA, N. B. [The origin of glacial relict amphipods in relation to the question of a postglacial connection between the white and Baltic Seas.] Uchenye Zapiski Karelo-Fin. Gosudarst. Univ. Biol. Nauki, **4**(3), 110–127 (1952).
43. MARSH, C. D. On the deepwater Crustacea of Green Lake, Wisconsin. Trans. Wisconsin Acad. Sci. **8**, 211–224 (1892).
44. MATHEWS, W. H. Glacial geology of British Columbia. Atlas of British Columbia Natural Resources, Map 4. B.C. Natural Resources Conference, Victoria (1956).
45. MOHR, J. L. Some observations on Arctic crustaceans and their associates: Notes on the fauna of Nuwuk Pond, Point Barrow. Arctic Research Lab. Notes, 1953–56, 15–18 (1953).
46. NORTHCOTE, T. G. and LARKIN, P. A. Indices of productivity in British Columbia lakes. J. Fisheries Research Can. **13**(4), 515–540 (1956).
47. NORTON, A. H. Proc. Portland Soc. Nat. Hist. **2**, 1–47 (1909).
48. ORTMAN, A. E. Higher crustaceans (Malacostraca). In Ward and Whipple's Freshwater biology. John Wiley & Sons, Inc., New York. 1918. Chap. 25. pp. 828–850.
49. PENNAK, R. W. Freshwater invertebrates of the United States. Ronald Press, New York. 1953.
50. RATHBUN, M. J. App. VI. List of the Crustacea on the Labrador coast. In Labrador: the country and its people. W. T. Grenfell. 1909. pp. 480–487.
51. RAWSON, D. S. The bottom fauna of Lake Simcoe and its role in the ecology of the lake. Univ. Toronto Studies Biol. Ser. No. 34; Publ. Ontario Fisheries Research Lab. No. 40, 1–177 (1930).
52. RAWSON, D. S. A comparison of some large alpine lakes of western Canada. Ecology, **23**, 143–161 (1942).
53. RAWSON, D. S. Preliminary biological and fisheries investigations of Reindeer Lake. Unpublished report to the Saskatchewan Department of Natural Resources, Regina (1942a).
54. RAWSON, D. S. Limnology and fisheries of five lakes in the upper Churchill drainage, Saskatchewan. Saskatchewan Dept. Nat. Resources Fisheries Rept. No. 3 (1957).
55. RAWSON, D. S. A limnological comparison of twelve large lakes in northern Saskatchewan. Unpublished manuscript (1959).
56. RICKER, W. E. The benthos of Cultus Lake. J. Fisheries Research Board Can. **9**, 204–212 (1952).
57. RIMSKY-KORSAKOV, V. N. The food of certain fishes of the Lake Champlain watershed. Suppl. 19th Ann. Rept. N. Y. Conserv. Dept. 88–104 (1930).

58. SAMTER, M. and WELTNER, W. Biologische Eigentümlichkeiten der *Mysis relicta*, *Pallasiella quadrispinosa* und *Pontoporeia affinis* erklärt aus ihrer eiszeitlichen Entstehung. Zool. Anz. **27**, 676-694 (1904).
59. SCHEFFER, V. B. and ROBINSON, R. J. A limnological study of Lake Washington. Ecol. Monogr. **9**, 95-143 (1939).
60. SCHMITT, W. L. The schizopod crustaceans of the Canadian Arctic Expedition, 1913-1918. Rept. Can. Arctic Exped. **7(B)**, 3-8 (1919).
61. SEGERSTRÅLE, S. B. Studien über die Bodentierwelt in sudfinnlandischen Küstengewässern. III. Zur Morphologie und Biologie des Amphipoden *Pontoporeia affinis*, nebst einer Revision des *Pontoporeia*-Systematik. Soc. Sci. Fennica, Commentationes Biol. **7**, 1-183 (1937).
62. SEGERSTRÅLE, S. B. The immigration history of the aquatic fauna of northern Europe. Soc. Sci. Fennica, Commentationes Biol. **16(16)**, 1-117 (1957).
63. SHOEMAKER, C. R. Amphipods. Rept. Can. Arctic Exped. 1913-1918, **7(E)**, 1-30 (1919).
64. SIBLEY, C. K. Fish food studies. Suppl. 21st Ann. Rept. N. Y. Conserv. Dept. 120-132 (1932).
65. SMITH, S. I. The Crustacea of the fresh waters of the United States. Rept. U. S. Comm. Fisheries for 1872-73, 637-665 (1874).
66. SMITH, S. I. List of the Crustacea dredged on the coast of Labrador by the Expedition under the direction of W. A. Stearns in 1882. Proc. U. S. Natl. Museum, **6**, 218-222 (1883).
67. SOUTHERN, R. and GARDINER, A. C. The seasonal distribution of the Crustacea of the plankton in Lough Derg and the River Shannon. Dept. Fisheries Ireland, Sci. Invest., 1-170 (1926).
68. SUMMERHAYES, V. S. and ELTON, C. S. Contributions to the ecology of Spitsbergen and Bear Island. J. Ecol. **6(2)**, 215-286 (1923).
69. STEPHENSEN, K. *Mysis oculata* in Greenland coastal waters. Medd. Grønland, **79(9)**, 1-20 (1933).
70. STEWART-HAY, R. K. Post-glacial dispersal in freshwater organisms. Summary in Proc. Can. Comm. for Freshwater Fisheries Research, **10(App. B)** (13) (1957). [Complete manuscript also examined.]
71. TATTERSALL, W. M. A review of the Mysidacea of the United States National Museum. Bull. U. S. Natl. Museum, **201**, 1-292 (1951).
72. THIENEMANN, A. *Mysis relicta*. Z. Morphol. Ökol. Tiere, **3**, 389-440 (1925).
73. THIENEMANN, A. Verbreitungsgeschichte der Süßwassertierwelt Europas. Die Binnengewässer, **18**, 1-809 (1950).
74. WILSON, C. B. The macroplankton of Lake Erie. App. 18th Ann. Rept. N. Y. Conserv. Dept. 67-76 (1929).
75. WILSON, J. T. et al. Glacial map of Canada. Geological Association of Canada, Ottawa. 1958.



# RECORDS OF CYAMUS BALAENOPTERAE BARNARD AND NEOCYAMUS PHYSETERIS (POUCHET), TWO SPECIES OF WHALE-LICE (AMPHIPODA), FROM THE NORTHEAST PACIFIC<sup>1</sup>

L. MARGOLIS

## Abstract

*Cyamus balaenopterae* Barnard from *Balaenoptera acutorostrata* and *Neocyamus physteris* (Pouchet) from *Physeter macrocephalus* are reported for the first time from the Pacific Ocean. This is the first record of a cyamid from *B. acutorostrata*.

*Cyamus balaenopterae* Barnard, 1931 and *Neocyamus physteris* (Pouchet, 1888) Margolis, 1955, collected from whales taken in the Northeast Pacific off the coast of British Columbia, have recently been identified by the author. These records represent the first of these two species in the Pacific Ocean. They were previously known from the Atlantic Ocean and they probably occur in the Antarctic. The distribution of *C. balaenopterae* and *N. physteris*, like other cyamid species, probably parallels that of their hosts.

## *Cyamus balaenopterae* Barnard

Nine specimens (five males and four females), which proved to be *C. balaenopterae*, were given to the author by Dr. C. G. Carl of the Provincial Museum, Victoria, British Columbia. The specimens were collected by Mr. Patrick M. Martin from a *Balaenoptera davidsoni* (= *B. acutorostrata*), the minke whale, little piked whale, or lesser rorqual, taken in Goletas Channel, British Columbia, on August 2, 1940. This is the first published account of cyamids occurring on *B. acutorostrata*.

The males measure 3.7 to 5.0 mm in total body length and the females measure 3.1 to 4.2 mm in length. Barnard (1, 2), who described this species from *Balaenoptera physalus*, the fin whale, and *Balaenoptera musculus*, the blue whale, from South Africa, gave the maximum length of males and females as 8.5 mm and 8 mm, respectively. The females in the present collection are obviously immature because the oostegites are far from completely developed to form the incubatory pouch. Because of the small size, the males probably also are immature. In spite of their immaturity, the specimens from *B. acutorostrata* exhibit all the distinguishing characteristics of *C. balaenopterae* as described by Barnard. I have also had the opportunity to confirm their identity by direct comparison with some of Barnard's specimens which were kindly loaned to me by Dr. Isabella Gordon of the British Museum (Natural History), where these specimens are presently housed. The presence of a prominent single accessory gill at the base of each gill in males, a readily observable feature even in the small males examined by the author, offers a good diagnostic character of *C. balaenopterae*. Other species of *Cyamus* possessing single, elongate, cylindrical gills on each side of pereopod segments

<sup>1</sup>Manuscript received August 7, 1959.

Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

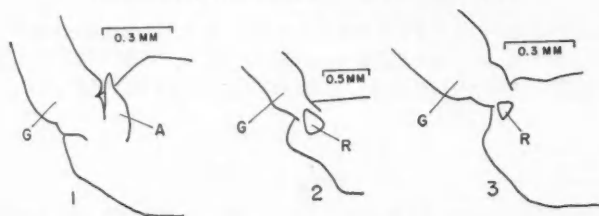


FIG. 1. *Cyamus balaenopterae*. Ventral view of gill and accessory gill of right side, segment 3, of male, 4.9 mm length.

FIG. 2. *Cyamus boopis*. Same, segment 4, of male, 6.5 mm length.

FIG. 3. *C. boopis*. Same, segment 3, of male, 4.6 mm length.

All figures drawn with the aid of a camera lucida. A=accessory gill. R=rudimentary accessory gill. G=gill.

3 and 4, except *Cyamus nodosus* Lütken, have biramous accessory gills. Single accessory gills (although less developed than those in *C. balaenopterae*) also are present in *C. nodosus*, but this species is unique in the possession of longitudinal grooves on the dorsal surface of pereaeon segments 3 to 7.

The single accessory gills of males of *C. balaenopterae* (Fig. 1) are unlike the single spine-like rudimentary accessory gills of juveniles of species whose adult males possess biramous accessory gills. For example, in juvenile males of *Cyamus boopis* Lütken (Figs. 2 and 3) of lengths up to about 7 mm the rudimentary gills are, in outline, roughly in the form of an equilateral triangle, have their apices directed towards the mid-line of the gills or somewhat posterior thereto, and do not extend beyond the lateral margins of the pereaeon segments; whereas in *C. balaenopterae* males the accessory gills are elongate, with their apices directed anterolateral to the gills, and extend beyond the margins of the pereaeon segments (at least in specimens of 4.2 mm or more in length).

Barnard's (1, 2) accounts of *C. balaenopterae* are the only published records of this species. Ohno and Fujino (7), and Kakuwa, Kawakami, and Iguchi (4) reported unidentified cyamids from *B. physalus* and *B. musculus* captured in the Antarctic from the 1946-47 to 1951-52 whaling seasons. For the 6-year period the mean incidence of cyamid infection of 7794 *B. physalus* and 3343 *B. musculus* was about 4.8% and 4%, respectively. In view of the host specificity generally exhibited by cyamids it is likely that the Antarctic specimens were *C. balaenopterae*.

Mr. G. C. Pike, biologist-in-charge of marine mammal investigations for the Fisheries Research Board Biological Station at Nanaimo, has informed me that he has observed cyamids on a small number of *B. physalus* taken by the commercial whaling fleet operating off the coast of British Columbia. Specimens have not been collected but again the species is likely to have been *C. balaenopterae*.

Because of the general lack of opportunity for transfer of cyamids from one whale species to another, I think that the occurrence of *C. balaenopterae* on three of the five species of *Balaenoptera* indicates that this cyamid was present on the common ancestor of *Balaenoptera* spp., and that speciation of the hosts has progressed without simultaneous speciation of their cyamid

parasites. Further field collections may also reveal the presence of this cyamid on *Balaenoptera borealis*, the sei whale, and *Balaenoptera brydei*, Bryde's whale.

### *Neocyamus physteris* (Pouchet, 1888)

Two males and one mature female of *N. physteris* were collected by Mr. Dale W. Rice, visiting biologist from the United States Fish and Wildlife Service, on April 7, 1959, from a female sperm whale (*Physeter macrocephalus*), length 34.5 ft, landed at the commercial whaling station at Coal Harbour, British Columbia.

*N. physteris* possesses many characters which distinguish it from other cyamids, amongst which the fasciculate gills are the most striking.

This species is the second cyamid to be recorded from *P. macrocephalus* (= *catodontis*) in the North Pacific. Margolis (5, 6) previously described *Cyamus catodontis* (= *C. boopis* var. *physteris* Pouchet, 1892) from this whale. Both species have been known for many years from sperm whales from the Atlantic Ocean. Clarke (3) recently briefly reviewed the published records of cyamids on sperm whales and noted the lack of records of *N. physteris* from the Pacific Ocean.

The history and synonymy of *N. physteris* have been reviewed by Margolis (6), who named the genus *Neocyamus* for *Cyamus physteris* Pouchet. The Atlantic localities from which this species definitely has been identified are the Azores and Bermuda (Pouchet (8, 9), Verrill (10)). Ohno and Fujino (7) and Kakuwa, Kawakami, and Iguchi (4) have recorded unidentified cyamids from 303 (about 20%) of 1542 sperm whales taken in the Antarctic during the whaling seasons of 1949-50 to 1951-52. In view of the occurrence of *N. physteris* in the Atlantic Ocean and Pacific Ocean, indicating a wide distribution of this species, some of the sperm whale cyamids from the Antarctic were probably *N. physteris*.

### References

1. BARNARD, K. H. Diagnoses of new genera and species of amphipod Crustacea collected during the 'Discovery' investigations, 1925-1927. *Ann. Mag. Nat. Hist.*, Ser. 10, 7, 425-430 (1931).
2. BARNARD, K. H. Amphipoda. *Discovery Repts.* 5, 1-326 (1932).
3. CLARKE, R. Sperm whales of the Azores. *Discovery Repts.* 28, 237-298 (1956).
4. KAKUWA, Z., KAWAKAMI, T., and IGUCHI, K. Biological investigation on the whales caught by the Japanese Antarctic whaling fleets in the 1951-52 season. *Sci. Repts. Whales Research Inst.*, Tokyo, 8, 147-213 (1953).
5. MARGOLIS, L. Three kinds of whale-lice (Cyamidae: Amphipoda) from the Pacific coast of Canada, including a new species. *J. Fisheries Research Board Can.* 11, 319-325 (1954).
6. MARGOLIS, L. Notes on the morphology, taxonomy and synonymy of several species of whale-lice (Cyamidae: Amphipoda). *J. Fisheries Research Board Can.* 12, 121-133 (1955).
7. OHNO, M. and FUJINO, K. Biological investigation of the whales caught by the Japanese Antarctic whaling fleets, season 1950-51. *Sci. Repts. Whales Research Inst.*, Tokyo, 7, 125-188 (1952).
8. POUCHET, G. Sur un nouveau *Cyamus* (*physteris*) parasite du cachalot. *Compt. rend.* 107, 698-699 (1888).
9. POUCHET, G. Contribution à l'histoire des Cyames. *J. Anat. et Physiol. norm. et pathol.* 28, 99-108 (1892).
10. VERRILL, A. E. Additions to the fauna of the Bermudas from the Yale Expedition of 1901, with notes on other species. *Trans. Conn. Acad. Arts Sci.* 11, 15-62 (1902).



## MOSQUITOES (DIPTERA: CULICIDAE) IN IRRIGATED AREAS OF SOUTHERN ALBERTA AND THEIR SEASONAL CHANGES IN ABUNDANCE AND DISTRIBUTION<sup>1</sup>

J. A. SHEMANCHUK

### Abstract

More than 95% of the mosquitoes in southern Alberta develop in waste irrigation water. Sixteen species of four genera in the subfamily Culicinae were identified, namely, *Anopheles earlei*, *Culex tarsalis*, *Culiseta inornata*, *Culiseta alaskaensis*, *Aedes vexans*, *A. cinereus*, *A. dorsalis*, *A. campestris*, *A. spencerii*, *A. flavescens*, *A. nigromaculis*, *A. riparius*, *A. sticticus*, *A. intrudens*, *A. cataphylla*, *A. melanion*; and two species in the subfamily Chaoborinae, namely, *Chaoborus americanus* and *C. flavicans*. Weekly index of the number of larvae and pupae as the average number per dip multiplied by the area in square yards, and total weekly trap catches of adults of the seven most common species were computed for each of three irrigation districts, representing distinctly different farming practices. Adults were more numerous in a sheltered than in an open site, the males noticeably more so than the females. Mosquitoes were more abundant in the older than in the newer irrigated districts, and improper farming and water-management practices favored increase in numbers, even in well-planned irrigation districts.

### Introduction

The mosquito problem in irrigated areas of southern Alberta first received attention in 1954 (3). Of the half million acres under irrigation in that region, almost all are south of the Red Deer River between the fourth and fifth meridians. In general the land, in the brown and dark-brown soil zones, is gently rolling, cut by deeply eroded coulees. The average annual precipitation is 11 to 15 in., and occasional droughts occur. In some areas, frequent hot dry winds (chinooks) cause high evaporation. The weather in 1956 and 1957 was normal, although the temperature was slightly higher each year than the mean for the period 1902-1954.

This is a report on studies conducted in 1956 and 1957 on seasonal changes in abundance and distribution of economically important mosquitoes in irrigated areas of Alberta. The studies included a comparison of effects of the various types of farming and water-management practices on the numbers of mosquitoes and of the effects of numbers of mosquitoes in irrigation systems of different ages.

### Materials and Methods

In 1956 two sampling areas were selected, each of 16 sq. miles. One area was an intensively irrigated region and the other an unirrigated grazing land. Mosquito larvae that developed in seepage and overflow water from irrigation canals cutting through the area on grazing land were disregarded.

All larval habitats in both areas were surveyed weekly. Mosquito numbers were estimated by counting the fourth-instar larvae and the pupae in each of 20 dips taken at random with a quart-size dipper from the periphery of each

<sup>1</sup>Manuscript received August 7, 1959.

Contribution from the Veterinary-Medical Entomology Section, Canada Agriculture Research Station, Lethbridge, Alberta; from a thesis submitted to the Faculty of Graduate Studies, University of Alberta, in partial fulfillment of the requirements for the M.Sc. degree, 1958.

pool remaining in the area each week. The index of numbers for each pool, adapted from work of the United States Public Health Service (1), was taken as the average number of larvae and pupae per dip multiplied by the surface area of the pool in square yards. The weekly number for each area was taken as the sum of the indices. Although this method provided only an estimate, it was rapid and required no cumbersome equipment. A representative sample of fourth-instar larvae was taken from each dip for identification. Total salt concentration and pH of the water from the larval habitats were measured by a conductivity bridge and a Beckman pH meter, respectively.

Two visual-attraction traps, developed by Haufe and Burgess (5), were operated 24 hours daily for samples of the adults in the irrigated area studied in 1956. One trap was placed in a sheltered area in the town of Brooks and another in an open pasture 1.5 miles to the east. Traps were not operated in the grazing area because electricity was not available.

In 1957 a 6-sq.-mile area was selected in each of three irrigation districts. Area 1 was in the Eastern Irrigation District near Rolling Hills, which is one of the oldest irrigation developments in Alberta. This area, in which the roads, irrigation supply, and drainage ditches are laid out in a grid system, has poor drainage facilities and the major enterprise was livestock production. Area 2 was in the Prairie Farm Rehabilitation Administration Irrigation Project near Hays, which had been irrigated for 6 years. This district, in which all of the irrigation facilities follow the contour of the land, has good drainage facilities, and the main enterprise was cereal crop production. Area 3 was in the Big Bend Project of the St. Mary's River Development near Taber, and it also had been irrigated for 6 years. This district, in which the irrigation facilities are laid out in a grid system as in area 1, had poor drainage facilities, and the main enterprise was row-crop rather than livestock production.

The index of numbers of larvae and pupae in 1957 were determined weekly by the method used in 1956. The adults were sampled by means of one visual-attraction trap set up in a farmyard in the three districts under comparable conditions.

### Species Present

In 1956 and 1957 the following species were found in the stages indicated:

|                                    | Larvae | Adults |   |
|------------------------------------|--------|--------|---|
|                                    |        | ♀      | ♂ |
| Culicinae                          |        |        |   |
| <i>Anopheles earlei</i> Vargas     | x      | x      | x |
| <i>Aedes vexans</i> (Meigen)       | x      | x      | x |
| <i>A. cinereus</i> (Meigen)        | x      | x      |   |
| <i>A. dorsalis</i> (Meigen)        | x      | x      | x |
| <i>A. campestris</i> Dyar and Knab | x      | x      | x |
| <i>A. spencerii</i> (Theobald)     | x      | x      | x |
| <i>A. flavescens</i> (Müller)      | x      | x      | x |
| <i>A. nigromaculis</i> (Ludlow)    | x      | x      | x |
| <i>A. riparius</i> Dyar and Knab   | x      | x      | x |
| <i>A. sticticus</i> (Meigen)       | x      | x      |   |
| <i>A. intrudens</i> Dyar           | x      | x      |   |

|   | Larvae | Adults |   |
|---|--------|--------|---|
|   |        | ♀      | ♂ |
| <i>Culicinae—Concluded</i>              |        |        |   |
| <i>A. cataphylla</i> Dyar               | x      | x      |   |
| <i>A. melanimon</i> Dyar                |        |        | x |
| <i>Culiseta inornata</i> (Williston)    | x      | x      | x |
| <i>C. alaskaensis</i> (Ludlow)          |        | x      |   |
| <i>Culex tarsalis</i> Coquillett        | x      | x      | x |
| <i>Chaoborinae</i>                      |        |        |   |
| <i>Chaoborus americanus</i> (Johannsen) | x      | x      | x |
| <i>C. flavicans</i> (Meigen)            | x      | x      | x |

Seven species of the subfamily Culicinae were regarded as common pests in irrigated areas of Alberta. In order of abundance these were *A. dorsalis*, *A. vexans*, *C. inornata*, *C. tarsalis*, *A. spencerii*, *A. campestris*, and *A. flavescens*. No other species appeared to be numerous enough to be classed as a serious pest. Some females of *A. melanimon* (4) were possibly identified as *A. dorsalis* because of the difficulty in distinguishing rubbed specimens.

### Notes on the Species

*A. dorsalis*.—Larvae were found in temporary pools in which the pH varied from 7.3 to 9.0 and the total salt concentrations reached 22,450 p.p.m. At total salt concentrations above 1900 p.p.m., larvae were found in association with *A. campestris* and, below this, in association with *A. vexans*, *A. flavescens*, *A. spencerii*, and *C. tarsalis*. Adults flew in great numbers, even in the short-grass pastures, at almost all times of the day.

*A. vexans*.—This species was one of the most annoying to man and livestock in the irrigated areas. Larvae were found in temporary pools in association with *A. dorsalis*, *A. flavescens*, *C. tarsalis*, and *C. inornata*. They were found in water with a pH between 7.7 and 8.5 and total salt concentrations between 499 and 1900 p.p.m. There were several broods during the season. Shelter belts and tall vegetation appeared to be the preferred resting places of the adults.

*C. inornata*.—In July, August, and September, larvae were found in almost every pool sampled. Adults did not attack man readily but were troublesome to livestock, especially after dark. This species overwinters as an adult and was taken in the visual-attraction traps as late as November 2.

*C. tarsalis*.—As with *C. inornata*, larvae of *C. tarsalis* were found in almost every pool in July, August, and September. They were found in association with larvae of *A. vexans*, *A. dorsalis*, and *C. inornata*. They tolerated total salt concentrations up to 1900 p.p.m. Females were observed to bite only after sunset, as observed by Hearle (6) but not by McLintock (7), who reported that they fed during the day.

*A. spencerii*.—This species was common in the irrigated areas but it was not as abundant as *A. vexans*, *A. dorsalis*, *C. tarsalis*, or *C. inornata*. It tolerated the same range of total salt concentrations as *A. vexans* and *A. flavescens*. Adults attacked at any time during the day when disturbed.

*A. campestris*.—Larvae were found in pools with high total salt concentration, usually between 1900 and 22,450 p.p.m., almost always in association with *A. dorsalis*. Adults bit whenever disturbed, even during the heat of the day.

*A. flavescens*.—Larvae were present throughout the season in varying numbers but only in pools with a total salt concentration below 1900 p.p.m. They appeared only in pools that dried out for at least 2 weeks before being reflooded. The adults seemed to prefer livestock and rarely attacked man.

*A. nigromaculis*.—Few larvae of this species were taken, in shallow depressions in pastures, and few adults were trapped. According to Rempel (9) this species is common in Saskatchewan. The reason for the low incidence in irrigated areas is not known.

*A. cinereus*.—Few larvae and adults were collected. Larvae were found only in pools surrounded by willow, *Salix* spp. The adults were not a serious pest in irrigated areas.

*A. earlei*.—A few larvae were taken in roadside ditches and in field ponds that were heavily overgrown with vegetation. They were found in the shaded portions of the pools where the water was rich in organic matter and algae. The author (unpublished data from 1952 and 1953) and Rempel (9) found this species in Saskatchewan in sluggish streams whose banks were overgrown with dense vegetation. Larvae were not found in the irrigation ditches sampled, probably because of the swift flow and the lack of overhanging vegetation. A few adults were taken in visual attraction traps in late July and early August.

*C. americanus* and *C. flavicans*.—*C. flavicans* was the more abundant of the two species. Larvae of the two species were associated with each other, mostly in semipermanent and permanent bodies of water. Adults were caught in abundance in the visual-attraction traps. In 1956, peak numbers of adults were trapped on June 24 and on August 20. Although adults of both species do not bite, they were a nuisance in dwellings and business places, being attracted to light in large numbers.

### Seasonal Changes in Abundance and Distribution

Differences in index of numbers of larvae and pupae in the two areas studied in 1956 occurred during the periods of irrigation, which extended from the first week in June to the last week in August (Fig. 1). Rainfall probably promoted development of some of the mosquitoes in the irrigated areas in 1956 during the irrigating season; this is indicated in the indices for the dry-land area for the first 3 weeks of August. Rainfall in June, July, and September was 2.23, 0.88, and 1.50 in., respectively, which was insufficient to promote development of mosquitoes. In August 2.19 in. of rain fell during the first 2 days and the pools that were formed were maintained long enough by a subsequent rainfall of 1.54 in. to allow larvae to mature. The small numbers of mosquitoes in May in the dry-land area was almost equal to that in the irrigated area. This was attributed to spring run-off rather than to the rainfall, which amounted to 0.75 in. in May, 1956.

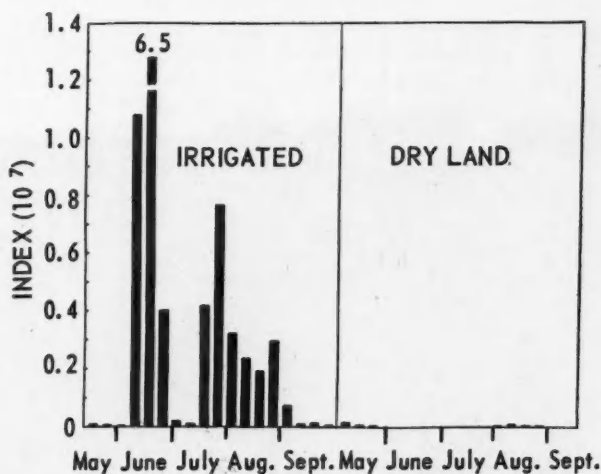


FIG. 1. Weekly indexes of mosquito larvae and pupae in irrigated and dry-land areas in the Eastern Irrigation District near Brooks, Alberta, in 1956.

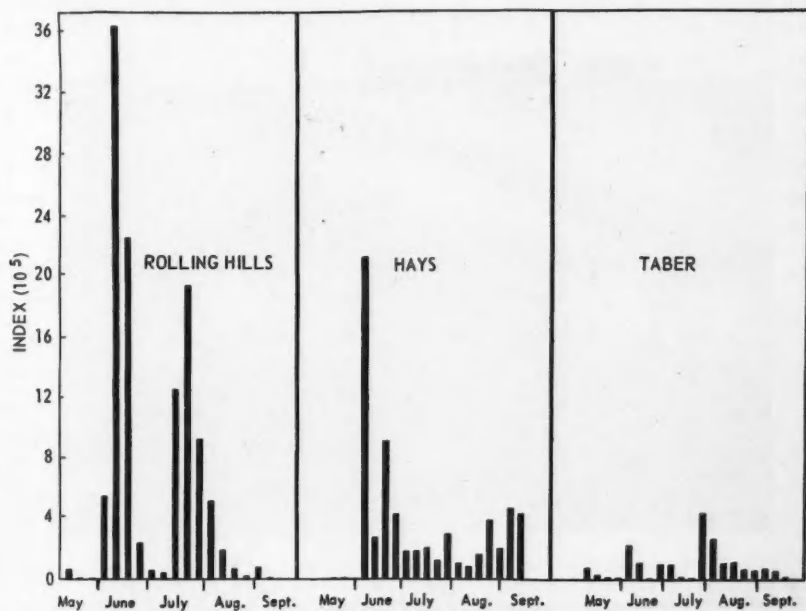


FIG. 2. Weekly indexes of the numbers of mosquito larvae and pupae in 1957 in representative areas of three irrigation districts of southern Alberta.



FIG. 3. A roadside ditch heavily overgrown with cattails (*Typhaceae*), as common in the Eastern Irrigation District.

FIG. 4. A seepage pool along a main irrigation canal near Rolling Hills, Alberta.

In the irrigated areas the number of larvae and pupae was highest during the second week in June, followed by lesser peaks during the third week in July and the third week in August (Fig. 1). The first peak occurred approximately 1 week after the initial major application of water to all crops. The second and third peaks followed the second and third applications of water, used to irrigate mainly the forage and pasture crops.

As in 1956, the numbers of larvae and pupae in 1957 were highest following irrigation in the three districts studied (Fig. 2). The numbers after spring runoff were insignificant compared with those after irrigation. The highest numbers occurred in the Rolling Hills district, where irrigation had been in operation for about 40 years. Tame hay and pasture fields common in that area were irrigated at least twice and sometimes three times in a season. These pastures were poorly drained and provided very suitable larval habitats for mosquitoes. Irrigation water from grain, hay fields, and pastures, allowed to accumulate in roadside ditches (Fig. 3), served as the main larval habitats. Seepage pools along main canals (Fig. 4), laterals, and supply ditches were also common in that district and promoted development of large numbers of *C. tarsalis* and *C. inornata*.

The major crops in the Hays area were wheat, oats, barley, and flax; in the Taber area, row crops such as sugar beets, corn, beans, canning peas, and potatoes. The numbers of larvae and pupae at Hays, though lower than at Rolling Hills, were higher than expected for an area with proper drainage facilities. Farmers in this community allowed water to remain on small areas of their fields. They evidently considered it more economical to sacrifice small portions of the low-priced crop than to expend the labor needed to control flooding. In contrast, the row crops in the Taber area were irrigated at regular intervals with specified amounts, thus controlling the amount of waste water and limiting mosquito breeding (Fig. 2). Since land for row crops required thorough levelling and preparation before irrigating, larval

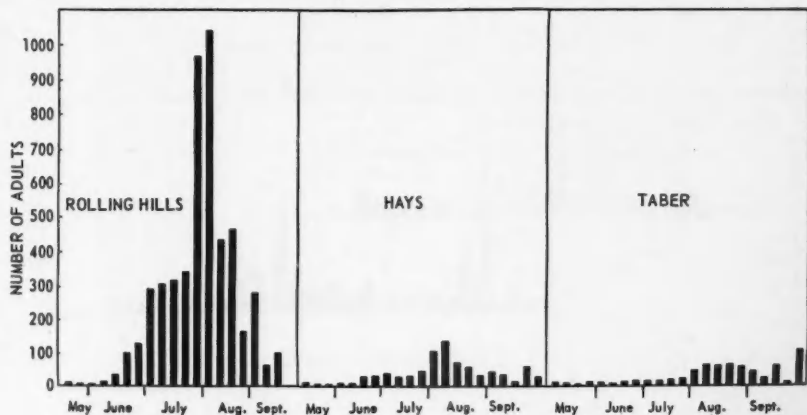


FIG. 5. Total weekly catches of adult mosquitoes in 1957 in visual-attraction traps at Rolling Hills, Hays, and Taber, respectively.

habitats are reduced further. Evidently the benefits from well-planned irrigation districts were vitiated by improper farming and water-management practices in the Hays district.

The numbers of adults in 1957 at Rolling Hills and Hays reached their peaks during the first and second weeks in August, respectively, and then declined gradually toward the end of the season (Fig. 5). In the Taber district a similar situation occurred except that the mosquitoes remained abundant throughout August, and the numbers in the third week in September and the first week in October were associated with the final applications of irrigation water to the sugar-beet crop. There was no similarity between the seasonal changes in the larval and adult abundance (Figs. 2 and 5).

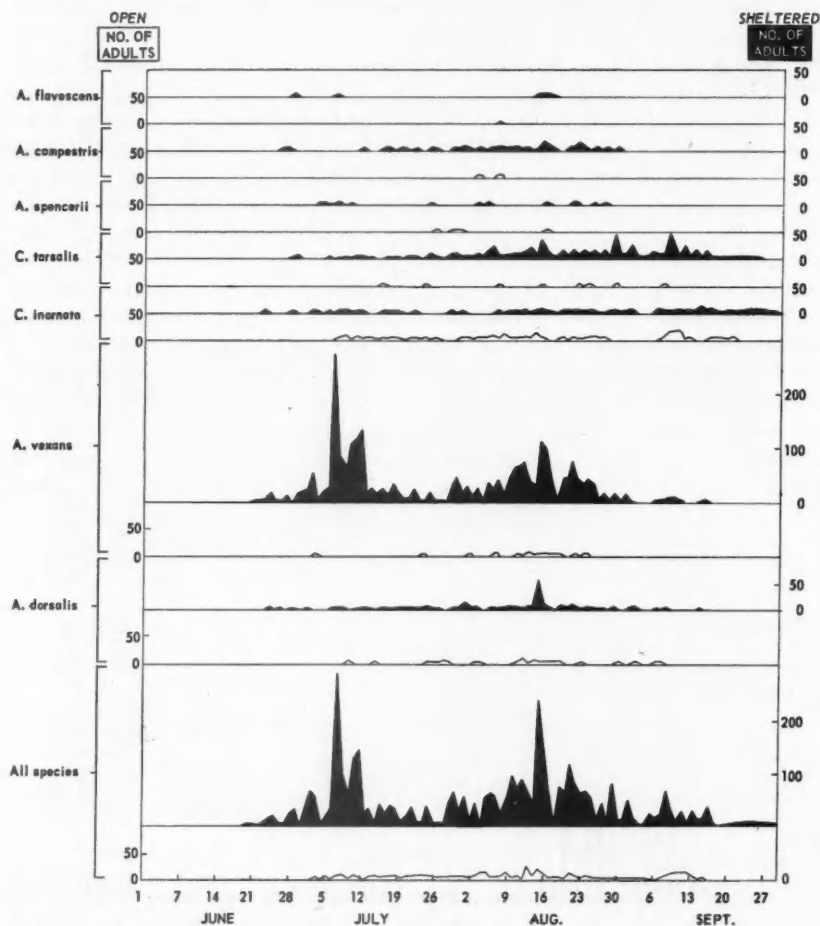


FIG. 6. Total daily catches of male mosquitoes in visual-attraction traps located in sheltered and open sites near Brooks, Alberta, in 1956.

Daily data from visual-attraction traps operated near Brooks in 1956 showed that male adults of all species were more abundant at the sheltered than at the open sites (Fig. 6). The species with the largest number of males was *A. vexans*, which showed peaks of abundance in the periods July 5-12 and August 7-23. Evidently a habitat with dense vegetation, especially shelter belts, was preferred by males. This preference has not been previously reported. The temperature and wind velocity would be lower and the humidity higher in dense than in sparse vegetation, making this habitat

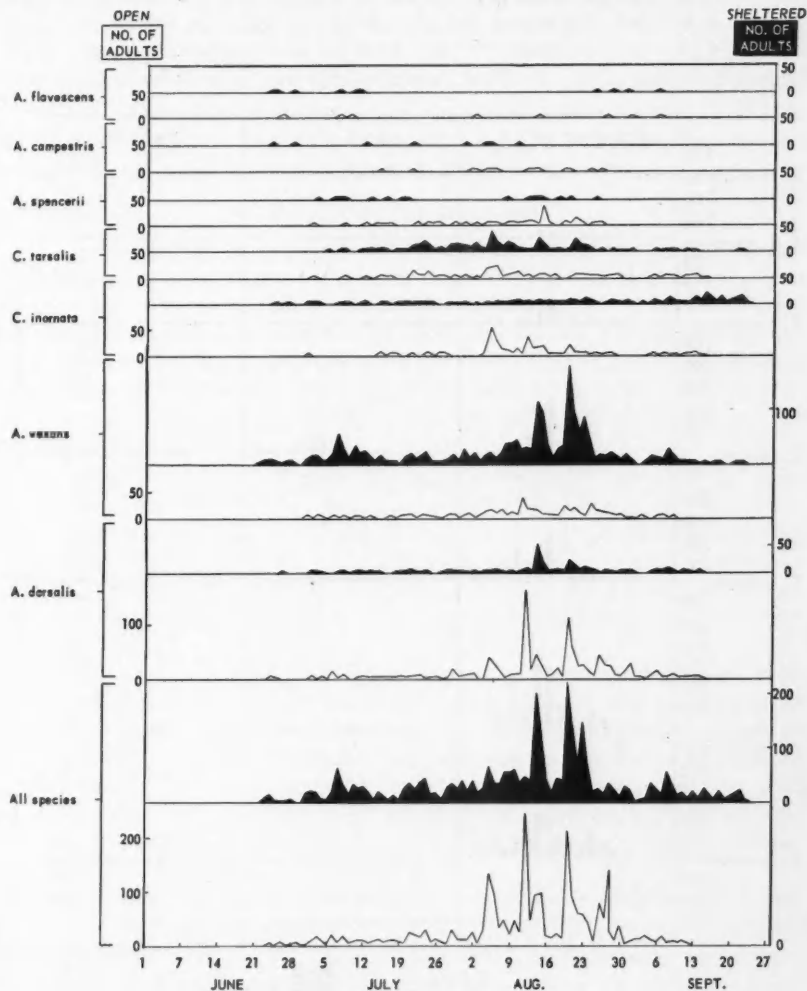


FIG. 7. Total daily catches of female mosquitoes in visual-attraction traps located in sheltered and open sites near Brooks, Alberta, in 1956.

more suitable to the resting males. West and Jenkins (14) reported that males of *A. communis* in the laboratory ingested plant nectars. Dense vegetation in sheltered farmyards, fields, and valleys of the irrigated districts includes more nectar-bearing plants than sparse prairie grass and thus serves as a source of food for the males.

The total daily catches of females were slightly higher in the sheltered than in the open site (Fig. 7). Females of *A. vexans*, *C. tarsalis*, and *A. spencerii* were distinctly more abundant in the sheltered site whereas *A. dorsalis* was more abundant in the open site. This distribution of the females can be attributed to their dispersion habits. After mating, the female has two important functions to fulfill: to seek food for ovarian development and to select oviposition sites. In these processes she travels over large areas and

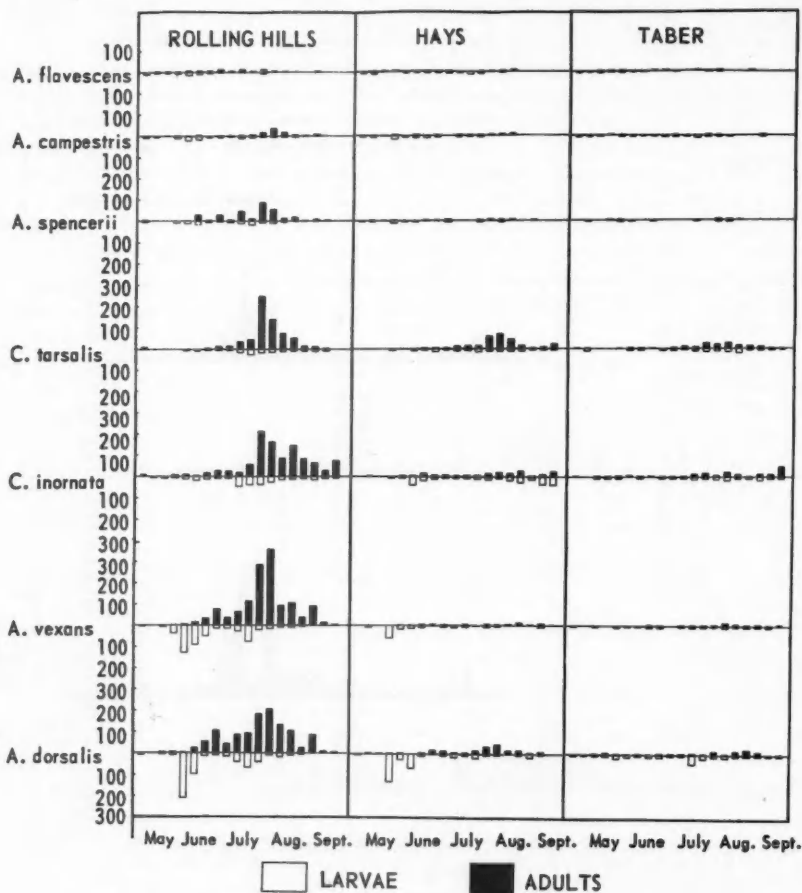


FIG. 8. Relative abundance throughout the season of larvae and pupae and of adults of seven common species of mosquitoes in the three irrigation districts studied in 1957.

thus is more likely to be sampled. Adjusting the index for the numbers of larvae and pupae and of adults to a common factor showed little quantitative relationship between them except that peaks for larvae and pupae were associated with later ones for adults (Fig. 8). The lack of quantitative relationship can be attributed to the following: (a) adult mosquitoes disperse over large areas so that the samples collected in traps might include the mosquitoes from larval habitats outside as well as inside the study area, (b) the life span of adults is longer (11, 12) than the developmental period of aquatic stages, so that there would be a greater chance of an adult being sampled, and (c) the drying-up of pools causes a high mortality in the larval and pupal stages.

*C. tarsalis*, *C. inornata*, and *A. dorsalis* were the three major species at Hays and Taber whereas *A. vexans*, *A. dorsalis*, *C. inornata*, and *C. tarsalis* were the major species at Rolling Hills (Fig. 8). The reason for the relatively low abundance of *A. vexans* at Hays and Taber is not known.

### Economic Implications

The areas studied in 1956 and 1957 were representative of the irrigated districts of southern Alberta. Results definitely showed that development of the mosquitoes was directly associated with irrigation. Large acreages are already utilized for production of feed for livestock. Pastures and hay fields are usually not so well prepared for flood-irrigating as those devoted intensively to cultivated crops. Thus the mosquito problem promises to become more acute unless steps are taken to level these fields and so destroy the breeding sites.

The gently rolling topography of the irrigated areas contributes to the development of mosquitoes since depressions that cannot be completely eliminated without considerable expense hold water long enough for mosquitoes to develop. Heavy deposits of alkali salts develop in many depressions (Fig. 9). These depressions are not only unsuitable for growing crops but also serve as reservoirs for runoff water from irrigated fields and are prolific larval habitats.

The data obtained show that large numbers of mosquitoes develop even in well-engineered districts like Hays and that farming as well as engineering practices play an important part in the prevention of mosquito breeding.

Excess irrigation water from fields, when allowed to accumulate in roadside ditches, not only provides suitable larval habitats for mosquitoes but also causes broken road shoulders (Fig. 10) and soft road beds. This can be overcome by better planning and construction of roads to provide better drainage.

The data from this study show that adult mosquitoes are most abundant in July and August, when farming operations in irrigated districts are at their peak. The inhabitants of the irrigated areas accept mosquitoes as an occupational hazard and adopt ways of living with them. Using repellents and head nets and timing work to the periods of least mosquito activity are some of the ways in which farmers cope with the problem.



FIG. 9. Alkali salt deposits resulting from repeated accumulations of water in poorly drained areas in the Eastern Irrigation District near Brooks, Alberta.

FIG. 10. Deterioration of roadbed as a result of irrigation-water accumulating in roadside ditches.

During the 2-year period of this study it was observed that livestock suffered most from mosquito attack. Cattle in mosquito-infested areas scarcely fed at night. They congregated in groups, stamping their feet, switching their tails, and snorting. This grouping may be a protective behavior against mosquitoes similar to that of northern reindeer described by Breev (2). Economic losses caused by mosquitoes in livestock production are possible through reduced grazing due to discomfort and annoyance as well as through loss of blood (8, 11). To date there is no practical way of protecting livestock in the field from mosquito attack other than destroying the pest.

Until we know more about the use of insecticides in irrigation water under southern Alberta conditions, chemical control of mosquitoes is not feasible. Large, sparsely settled areas cannot bear the cost of an annual chemical control program, although space and residual sprays used about dwellings and farmsteads give local protection.

Of the four most abundant species breeding in irrigation water, *C. tarsalis* and *C. inornata* have been incriminated as vectors of western equine encephalitis from wild host to man (10). It is therefore remarkable that only six cases of human encephalitis have been reported from Alberta. Rempel (10) reported 543 human cases from Saskatchewan in 1941, of which 44 were fatal. Tests of almost all pooled samples of *C. tarsalis* adults collected in 1955 in the Milk River Valley of Montana, approximately 200 miles south of the areas surveyed in this study, were positive for western equine encephalitis (13). Therefore, there may be a similar incidence of the virus in southern Alberta. Evidently the question of mosquito-borne diseases warrants further study in Alberta, especially in irrigated districts.

### Acknowledgments

The author wishes to express thanks to the following: Dr. Brian Hocking, Department of Entomology, University of Alberta, for his review of the manuscript; H. Likuski and W. Klassen, student assistants, Veterinary-Medical Entomology Section, Lethbridge, for able assistance in the field; the staff of the Alberta Horticultural Station, Brooks, for laboratory facilities in 1956; and the staff at the Prairie Farm Rehabilitation Administration headquarters, Hays, for laboratory facilities in 1957.

### References

1. ANONYMOUS. First progress report, mosquito investigations, Milk River Irrigation Project, Montana. U. S. Dept. (of) Health, Education, and Welfare, Public Health Service, Communicable Disease Center, Technology Branch April, 1953. (Mimeographed.)
2. BREEV, K. A. On the behavior of blood-sucking flies and gad flies during their attack on northern reindeer and the response reaction of the reindeer. II. Gregariousness of reindeer as a protective measure against attack from blood-sucking insects (In Russian). *Zbornik paristol.* **13**, 343-354 (1951).
3. BROWN, J. H. The mosquito problem in relation to irrigation development in Alberta. *Can. J. Public Health*, **45**, 93-99 (1954).

4. BURGESS, L. Note on *Aedes melanimom* Dyar, a mosquito new to Canada (Diptera: Culicidae). *Can. Entomologist*, **89**, 532 (1957).
5. HAUPE, W. O. and BURGESS, L. Design and efficiency of mosquito traps based on visual response to patterns. To be published.
6. HEARLE, E. The mosquitoes of the lower Fraser Valley and their control. *Can. Nat. Research Council Rept. No. 17*. Ottawa. 1926.
7. McLINTOCK, J. The mosquitoes of the greater Winnipeg area. *Can. Entomologist*, **76**, 89-104 (1944).
8. MUNROE, E. R. and HOFFMAN, R. A. The volume of bovine blood ingested by *Aedes nigromaculis*. *Calif. Vector News*, **3**, 19 (1957).
9. REMPEL, J. G. A guide to the mosquito larvae of Western Canada. *Can. J. Research, D*, **28**, 207-248 (1950).
10. REMPEL, J. G. The mosquitoes of Saskatchewan. *Can. J. Zool.* **31**, 433-509 (1953).
11. STAGE, H. H. and YATES, W. W. Some observations on the amount of blood engorged by mosquitoes. *J. Parasitol.* **22**, 298-300 (1936).
12. STAGE, H. H., GJULLIN, C. M., and YATES, W. W. Flight range and longevity of flood water mosquitoes in lower Columbia River Valley. *J. Econ. Entomol.* **30**, 940-945 (1937).
13. WEST, A. S. The potential of mosquito-borne diseases in Canada. *Proc. 43rd Meeting New Jersey Mosquito Exterm. Assoc.* 1956.
14. WEST, A. S. and JENKINS, D. W. Plant feeding habits of northern mosquitoes studied with radioisotopes. *Mosquito News*, **11**, 217-219 (1951).

## ARTIFICIAL DIETS FOR THE HOUSE CRICKET, *ACHETA DOMESTICUS* (L.)<sup>1</sup>

J. E. MCFARLANE,<sup>2</sup> BARBARA NEILSON,<sup>3</sup> AND A. S. K. GHOURI<sup>4</sup>

### Abstract

Nymphs of *Acheta domesticus* (L.) will grow well on a diet containing casein, glucose, cholesterol, a mixture of inorganic salts, and a mixture of the known B-vitamins. Growth on other artificial diets is reported and discussed.

### Introduction

The literature on insect nutrition has been abundantly and recently reviewed (9). The only previous study which has been made of cricket nutrition is that of Chauvin (1), who found that nymphs of *Acheta domesticus* (L.) would develop to the adult stage when fed a diet consisting of 43% casein, 43% glucose, 10% powdered dry yeast, 4% McCollum's salt mixture, and 2% cholesterol or ergosterol, although the adult weight obtained was less than that of nymphs reared on lettuce. He further found that a sterol was essential for growth.

Our attempts to rear the nymphs of *A. domesticus* on Chauvin's diet have not been very successful. We have found that the addition of a mixture of B-vitamins to Chauvin's diet is essential for good growth and survival of the nymphs of this species, and that the yeast may be adequately substituted by a mixture of the known B-vitamins.

### Materials and Methods

Methods of culturing *A. domesticus* have been previously described (5). The Canadian strain was used in the present experiments.

The method used for testing the diets was an adaptation of one described before which was used to determine the effect of temperature on nymphal growth (5). Ten nymphs were reared in a 16-oz ointment jar. A shell vial of 32-ml capacity, filled with distilled water and plugged with aseptic cotton, was placed, inverted, in the jar; this served as the water supply. The dry diet was placed in a shallow boat made out of filter paper, and the boat was set on the bottom of the jar diametrically opposite to the moist cotton of the water vial; in no instance did a diet become moistened by water from the vial. The surface area was increased with a 1½×6 in. strip of folded filter paper. At weekly intervals, the nymphs were transferred to clean jars containing fresh diet, clean filter paper, and clean water vials.

Sixty insects less than 24 hours old were originally placed on each diet under test. The results reported here are from a single experiment using nymphs of one brood.

<sup>1</sup>Manuscript received September 15, 1959.

Contribution from the Department of Entomology, Macdonald College, McGill University, Macdonald College P.O., Que. Supported in part by a Quebec Agricultural Grant.

<sup>2</sup>Department of Entomology, Macdonald College.

<sup>3</sup>Terrasse Vaudreuil, Que.

<sup>4</sup>Department of Plant Protection, Karachi, Pakistan.

TABLE I  
Composition of the three basic diets. Figures represent parts in diet

|                  | Diet 1 | Diet 2 | Diet 3 |
|------------------|--------|--------|--------|
| D(+) dextrose    | 20     | 45     | 20     |
| Casein           | 40     | 45     | 40     |
| Yeast            | 10     | 10     |        |
| Cholesterol      | 1      | 1      | 1      |
| Salt mixture     | 2      | 2      | 2      |
| Cellulose powder | 30     |        | 30     |
|                  | 103    | 104    | 93     |

The temperature in the incubator was  $33 \pm 1^\circ \text{C}$  and the relative humidity  $55 \pm 5\%$ .

The composition of the three basic diets is given in Table I.

Diets were prepared in the following way: casein, glucose, and salts (also yeast, when a constituent) were mixed together, the vitamins were added in aqueous solution, and the diet was then dried in a desiccator and ground in a mortar; the cellulose powder was then added; and finally the cholesterol in chloroform solution. The vitamin mixtures were added to 103 g of diet 1, 104 g of diet 2, and 93 g of diet 3.

Three vitamin mixtures were used. Vitamin mixture A consisted of the following vitamins (mg): thiamin 2.5, riboflavin 1.25, nicotinic acid 5.0, pyridoxine 1.25, calcium pantothenate 2.5, choline chloride 50, inositol 25, folic acid 0.25, biotin 0.025, and *p*-aminobenzoic acid 2.5. This mixture contains the vitamins in the same proportions as the vitamin mixture of Fraenkel and Blewett (3), and the resulting concentrations of the vitamins in the diets are approximately the same as they used. Vitamin mixture B was the same as A, except that the *p*-aminobenzoic acid was omitted. Vitamin mixture C contained double the amounts of vitamins as B.

All purified chemicals were obtained from the Nutritional Biochemicals Corp. (Cleveland, Ohio) and included 'Vitamin-test' casein, 'Alphacel' cellulose powder, brewer's yeast, and the salt mixture U.S.P. XIV. The 'Baby rabbit pellets' were obtained from Ogilvie Flour Mills, Montreal.

Nymphs were weighed after 14 and after 28 days on the diets. All adults were weighed within 24 hours of their reaching that stage.

### Results and Conclusions

The results are presented in Tables II and III.

Growth on the rabbit pellets was much better, as regards adult weight obtained, than growth on any of diets using purified chemicals.

The differences in the average duration of the nymphal stage are significant (at  $P = 0.05$ ) between males and females reared on diets II and IV, indicating that wheat germ oil improves the rate of growth; and between females (but not males) reared on diets II and III, indicating a beneficial effect of *p*-aminobenzoic acid. The differences in the average weight of adults obtained

TABLE II

Survival and average weight of nymphs after 14 and 28 days

| Exptl.<br>diet | Composition                      | After 14 days |                 | After 28 days |                 |
|----------------|----------------------------------|---------------|-----------------|---------------|-----------------|
|                |                                  | No.           | Av. wt.<br>(mg) | No.           | Av. wt.<br>(mg) |
| I              | Basic diet 1                     | 50            | 9.8             | 42            | 32.1            |
| II             | Diet I + vitamin mixture B       | 49            | 22.2            | 46            | 114             |
| III            | Diet I + vitamin mixture A       | 52            | 22.8            | 46            | 138             |
| IV             | Diet II + 1 part wheat germ oil  | 53            | 24.0            | 47            | 153             |
| V              | Diet II minus cholesterol        | 30            | 3.4             | 9             | 11.5            |
| VI             | Basic diet 2                     | 46            | 6.8             | 40            | 21.9            |
| VII            | Diet VI + vitamin mixture B      | 50            | 22.4            | 43            | 99.2            |
| VIII           | Basic diet 3 + vitamin mixture B | 45            | 15.0            | 40            | 52.1            |
| IX             | Basic diet 3 + vitamin mixture A | 47            | 13.8            | 43            | 50.6            |
| X              | Basic diet 3 + vitamin mixture C | 52            | 14.8            | 45            | 76.3            |
| XI             | Rabbit pellets                   | 53            | 22.8            | 44            | 202             |

TABLE III

Number and average weight of adults obtained, average duration of nymphal stage, and % survival

| Exptl.<br>diet No. | Adult males obtained |   |                 | Adult females obtained |   |                 |               |
|--------------------|----------------------|---|-----------------|------------------------|---|-----------------|---------------|
|                    | No.                  | Av.<br>duration<br>nymphal<br>stage<br>(days) | Av. wt.<br>(mg) | No.                    | Av.<br>duration<br>nymphal<br>stage<br>(days) | Av. wt.<br>(mg) | %<br>survival |
| I                  | 8                    | 78  | 225             | 13                     | 76  | 244             | 35            |
| II                 | 20                   | 48*   | 272†            | 22                     | 45  | 297             | 72            |
| III                | 23                   | 45*   | 266†            | 21                     | 41  | 290             | 73            |
| IV                 | 20                   | 43†   | 264*            | 25                     | 41  | 334             | 75            |
| V                  | 0                    | —   | —               | 0                      | —   | —               | 0             |
| VI                 | 10                   | 73  | 190             | 6                      | 70  | 199             | 27            |
| VII                | 20                   | 50*   | 256†            | 17                     | 46  | 252             | 62            |
| VIII               | 13                   | 67  | 229             | 18                     | 60  | 219             | 52            |
| IX                 | 15                   | 67  | 243             | 11                     | 59  | 211             | 43            |
| X                  | 21                   | 50†   | 218*            | 17                     | 48  | 256             | 63            |
| XI                 | 21                   | 41*   | 351*            | 23                     | 37  | 393             | 73            |

\*Significantly different from female average at  $P = 0.05$ .†Not significantly different from female average at  $P = 0.05$ .

are significant between females reared on diets II and IV, but not between males; and the differences between both males and females reared on diets II and IV are not significant.

Growth on a diet containing yeast is better than on a diet lacking yeast (diets II and X), but the difference in the average duration of the nymphal stage of males is not significant.

Growth on diets II (with cellulose) and VII (no cellulose) is about the same, except for a significant difference in the adult female weight. Cellulose is apparently not utilized by the insect, as it has not been possible to demonstrate a cellulase or cellobiase in the gut of the adult insect. The excreta of

nymphs reared on diets which do not contain cellulose are semiliquid, whereas nymphs on diets containing cellulose void excreta which are pellet-like and dry.

Cholesterol is required by *A. domesticus* (diet V), as was found by Chauvin (1).

### Discussion

These results are not comparable in detail with Chauvin's (1), as he presents no data on the duration of the nymphal stage or on the adult weight obtained. As the vitamin content of casein and of yeast is widely variable, this probably accounts for our failure to get good growth with a diet (VI) similar to Chauvin's. Indeed the results presented here with diet VI are much the best we have obtained with this or similar ones, through several changes of casein and yeast. It is also possible that racial and perhaps species differences are involved, in view of the complex taxonomic picture that crickets present, in which reproductive isolation exists without marked morphological differences being apparent (4).

The B-vitamins are required in greater concentrations in the diet of the house cricket than in the diets of stored products insects (3). The house cricket is an omnivorous insect (5), however, and ground cereal seeds of many kinds, supplemented with yeast, do not provide for growth as good as that on materials of animal origin, such as skim milk or fish meal, supplemented with yeast (McFarlane and Ghouri, unpublished data). Growing plants may, however, be more satisfactory. A report on the specific vitamin requirements of the house cricket is in preparation (7).

Lipid other than cholesterol appears to promote growth of *A. domesticus*, and in previous work (5) we used a diet containing more than 5% corn oil, with no noticeably bad effects.

The diet II of this article has been found to provide for good growth and wing development of *Grylloides sigillatus* (Walker) (McFarlane, unpublished data) and it is quite possible that nutritional studies with this insect may go far towards explaining wing polymorphism in this species (6) and in the Orthoptera in general (2, 8).

### References

1. CHAUVIN, R. Les stérols dans le régime alimentaire de certains Orthoptères. *Compt. rend.* **229**, 902 (1949).
2. CHOPARD, L. In *La biologie des Orthoptères*. Edited by Paul Lechavalier et fils, Paris (1938).
3. FRAENKEL, G., BLEWETT, M., and COLES, M. The nutrition of the mealworm, *Tenebrio molitor* L. (Tenebrionidae, Coleoptera). *Physiol. Zool.* **23**, 92-108 (1950).
4. GHOURI, A. S. K. and MCFARLANE, J. E. Reproductive isolation in the house cricket (Orthoptera: Gryllidae). *Psyche*, **64**, 30-36 (1957).
5. GHOURI, A. S. K. and MCFARLANE, J. E. Observations on the development of crickets. *Can. Entomologist*, **90**, 158-165 (1958).
6. GHOURI, A. S. K. and MCFARLANE, J. E. Occurrence of a macropterous form of *Grylloides sigillatus* (Walker) (Orthoptera: Gryllidae) in laboratory culture. *Can. J. Zool.* **36**, 837-838 (1958).
7. MCFARLANE, J. E. and RITCHOT, C. Vitamin requirements of the house cricket. To be published.
8. SELLIER, R. Recherches sur la morphogénèse et le polymorphisme alaires chez les Orthoptères Gryllides. *Ann. sci. nat. Zool.* **16**, 595-739 (1954).
9. WHITELOCK, O. v. St. (Editor). Axenic culture of invertebrate metazoa: a goal. *Ann. N.Y. Acad. Sci.* **77**, 25-406 (1959).

# POPULATION DYNAMICS OF THE LODGEPOLE NEEDLE MINER, *RECURVARIA STARKI* FREEMAN, IN CANADIAN ROCKY MOUNTAIN PARKS<sup>1</sup>

R. W. STARK<sup>2</sup>

## Abstract

The lodgepole needle miner, *Recurvaria starki* Freeman, has been studied intensively since 1948. Life tables, survival, and death-rate curves show clearly that there are five periods in the 2-year life cycle of the needle miner during which extensive mortality may occur: (1) between egg formation and oviposition; (2) between oviposition and larval establishment; (3) during the first larval hibernation; (4) during the second larval hibernation; (5) during the spring of moth emergence. Population success is also undoubtedly affected by conditions during the adult life.

Population sampling has shown that the outbreak has declined since 1948. Defoliation and increment studies have shown that the period of greatest defoliation occurred from 1940 to 1944 and that the outbreak probably began in the late 1930's. The major cause of the decline was severe winter temperatures, probably during the coldest month. Parasitism was not an important factor in the outbreak decline, apparently because it was controlled in the same manner as the host, by winter temperatures. Other natural control factors are discussed as well as the possible effects of climatic factors on oviposition and fecundity.

A detailed survey of weather records since 1920 and yearly averages since 1885 suggest that release of the needle miner population was due to a warming trend in the climate of western Canada. This trend began in the late 1930's, reached a peak in the mid-1940's, and has declined since that time. The warming trend in northern latitudes has been noted by other authors and is substantiated by weather records of this region. It is further postulated that the climate of the Canadian Rocky Mountains is generally too severe for an outbreak of the lodgepole needle miner to be prolonged.

## Introduction

The lodgepole needle miner, *Recurvaria starki* Freeman,<sup>3</sup> is a defoliator which attracted attention because of its increase in abundance in the Canadian Rocky Mountains during the 1940's. The forests attacked by this insect cover a vast western watershed and are the main forest stands in Banff, Yoho, Kootenay, and Jasper national parks.

The outbreak on which these studies are based was more extensive and severe in Banff Park than in other areas. *Pinus contorta* ssp. *latifolia* (Engelm. ex Wats.) is the sole host. The parks are similar physiographically: there is a relatively narrow valley in each with steep, high sides formed by mountain ranges of altitudes up to 10,000 ft. The direction of the valley is northwest in Banff, south in Kootenay, and west in Yoho. Timberline varies from 6500 ft to 8000 ft, being about 7200 ft in the Banff area. Valley bottom varies in

<sup>1</sup>Manuscript received June 2, 1959.

Contribution No. 564, Division of Forest Biology, Research Branch, Department of Agriculture, Ottawa, Canada. Ph.D. thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, February 24, 1958.

<sup>2</sup>Forest Biology Laboratory, Calgary, Alberta.

<sup>3</sup>Publications prior to 1953 referred to this needle miner as *Recurvaria milleri* Busck and from 1953 to 1957 as *Recurvaria* sp.

altitude from 4000 ft at the eastern approaches to about 5000 ft at the Continental Divide. The main forest cover in the outbreak area consists of lodgepole pine stands of fire origin with an understory of spruce. Various age classes are represented but most stands are at least 80 years old.

The affected stands are adjacent to other extensive lodgepole pine stands of the eastern slopes of the Rocky Mountains in Alberta which form an important part of the province's forest industry. These considerations made it important to analyze the factors responsible for the increase in needle miner populations in the region, to explain zones of abundance within the outbreak, and to determine the factors which may limit outbreaks to the region recently affected. Since the late 1940's, needle miner populations have declined until at the present time (1959) high populations do not exist. This leads to the question whether such populations may recur although increment studies indicate that no previous outbreaks have occurred in this region during the life of the present timber stands. This paper is the third in a series dealing with various aspects of needle miner populations. The first (40) discussed sampling methodology and the second (42), the relation of climate to larval mortality of the needle miner. This paper describes the decline of the outbreak, outlining the control factors involved, and proposes a theory to explain the course of the outbreak. A subsequent paper describing the parasite complex and a discussion of parasite epidemiology is planned.

The needle miner in the Canadian Rockies has a 2-year life cycle (39). In the even-numbered years the adults emerge in July; eggs are laid in late July and August and hatch in August and September. Each larva immediately enters a needle in which it spends the first winter. The following spring, the miner commences to feed in late April or May, depending on spring weather, and completes the mining of the first needle. Transfer to a second needle takes place in mid-summer; climatic conditions affect the time and duration of the transfer period considerably. The larva overwinters in the second needle and the following spring, again an even-numbered year, transfers to a third needle. It completes mining by early June, pupates within the last mine, and the moth emerges 3 to 4 weeks later.

There have been three instances of out-of-phase populations. These are included in the life tables for 1958 as they were apparently part of the original established population. This proportion of the population was small and restricted in distribution, and no continuity of generations has been observed.

The outbreak was first noticed in June, 1942, on an area of approximately 50 square miles in Banff Park where it joins Kootenay Park at Vermilion Summit. The attack was largely confined to elevations between 5000 and 6500 ft (5). By 1944 it had spread into Yoho and Kootenay parks for short distances (18) and by 1946 it was estimated to cover 300 sq. miles, mostly in Banff Park (14). Populations had also increased in numbers below the 5000 ft levels, extending into the valley bottom (13). The outbreak covered an area of 400 sq. miles by 1948 (21) and a second outbreak in Jasper National Park

was reported on the slopes below Mount Edith Cavell (22, 37). This was entirely separate from the more southerly outbreak and was considered autochthonous (8).

### Life Tables for the Lodgepole Needle Miner

The formulation and use of life tables for needle miner epidemiological studies have been described (40). The sampling unit upon which the life tables were based is the number of individuals per 5-year branch tip (38). This is expressed under the  $lx$  column as the number of individuals per 100 branch tips. Six sampling periods: one egg, four larval, and one pupal, were deemed suitable to assess the course of a single generation from the time of oviposition to moth emergence (40). Each stage is sampled until the mean number of needle miners found, regardless of condition, agrees with the estimate from the previous sample (error limits,  $\pm 10\%$  of the mean). The total number of dead larvae is derived by subtracting the number of live larvae found (in sample) from the previous sample. The number attributed to any single mortality factor is calculated from the percentage mortality caused by that factor. The estimates are rounded and apportioned to the known mortality factors so that the life table is subtractive throughout. The following four areas are being sampled regularly for additional life table data:

(1) *Mount Eisenhower, Banff National Park*.—The sample area is located 22 miles northwest of the town of Banff, Alberta, on the east side of the Bow Valley. The elevation sampled is 5400 ft, 750 ft above valley bottom (Tables I and II, Figs. 1 and 2).

(2) *Massive Range, Banff National Park*.—This area is located about 11 miles northwest of Banff on the west side of the Bow Valley. The stands sampled are at approximately 5400 ft elevation, 600 ft above valley bottom (Tables III and IV, Figs. 3 and 4).

(3) *Mount Girouard, Banff National Park*.—The area sampled is located about 9 miles northeast of Banff on the south side of Lake Minnewanka. The samples are taken at approximately 6000 ft, 700 ft above valley bottom (Tables V and VI, Figs. 5 and 6).

(4) *Mount Cathedral, Yoho National Park*.—This area is located on a north-facing slope about 250 ft from valley bottom, elevation 4950 ft. It is approximately 6 miles from the Continental Divide (Tables VII and VIII, Figs. 7 and 8).

Two methods have been used in attempts to determine moth fecundity: controlled matings and moth dissections. Attempts to mate needle miner moths have been only partially successful; perhaps flight is necessary prior to copulation. Moth dissections made over the period 1950 to 1958 gave some measure of the minimum number of eggs that needle miner moths are capable of laying. The number of eggs used to calculate the expected number in all areas was 12 to 37. This range encompasses all previous estimates but observations lead the author to believe that the actual numbers laid are in the bottom of this range.

TABLE I<sup>1</sup>Life table for the 1954-1956 generation of needle miner,  
Mount Eisenhower (5400-ft elevation)

| <i>X</i>  | <i>lx</i>  | <i>dxF</i>                            | <i>dx</i>                | 100 <i>qx</i> |
|---|------------|---------------------------------------|--------------------------|---------------|
| <i>X</i> <sub>1</sub> : eggs, July, 1954  | 4700       | Needle drop and unknown<br>Resination | 3586<br>0                | 76.30         |
|   |            |                                       | 3586                     | 76.30         |
| <i>X</i> <sub>2</sub> : I and II instars, Sept., 1954                             | 1114       | Winter mortality                      | 409                      | 36.68         |
| Extra: Dec. 14, 1954  | 705        | Winter mortality                      | 219                      | 31.06         |
|   |            | Spring mortality                      | 70                       | 9.93          |
|   |            | Out-of-phase larvae                   | 0                        | —             |
|   |            |                                       | 289                      | 40.99         |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars,<br>July, 1955 | 416        | Winter mortality                      | 143                      | 34.37         |
|   |            | Spring mortality                      | 0                        |               |
|   |            | Bird predation                        | 0                        |               |
|   |            | Unknown                               | 8                        | 1.98          |
|   |            |                                       | 151                      | 36.35         |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1956                               | 265        | Larval parasitism                     | 160                      | 60.38         |
| <i>X</i> <sub>6</sub> : Pupae, June, 1956   | 105        | Pupal parasitism                      | <1                       | 0.45          |
|   |            | Unknown pupal mortality               | 26                       | 24.76         |
|   |            | Moths trapped in needles              | 0                        |               |
|   |            |                                       | 26+                      | 25.21         |
| Emergents   | 79         |                                       |                          |               |
| Sex ratio 52:48   | 41F 38M    |                                       |                          |               |
| Generation mortality  |            |                                       | 4621                     | 98.32         |
| Expected number of eggs   | = 456-1406 |                                       |                          |               |
| Measured number   | = 378      |                                       | Population trend = 8.0%  |               |
| Number of larvae established  | = 308      |                                       | Population trend = 27.5% |               |

<sup>1</sup>Table headings: *X*, stage at which sample is taken; *lx*, the number surviving at the beginning of the stage noted in the *X* column; *dxF*, the mortality factor responsible for *dx*, the number dying within the interval between successive samples; 100 *qx*, percentage mortality. The 'population trend' is the percentage calculated by comparing the number of eggs or larvae of the new generation to those at the beginning of the *lx* column.

## Natural Control Factors

### CLIMATIC FACTORS

#### (1) Winter Mortality

"Cold death" of insects has been extensively reported on in the literature, including various examples of the control of insect populations (3, 6, 10, 17, 20, 23, 30). Limited tests on lodgepole needle miner larvae in the laboratory indicated that they are extremely cold-hardy, even in the immature stages. Tests on first-instar larvae, in August and September, demonstrated that they could withstand temperatures of 21° F for periods up to 24 hours; temperatures of 0° F caused no mortality in 1 hour but almost 100% mortality in 24 hours. Dissections of third- and fourth-instar larvae in November

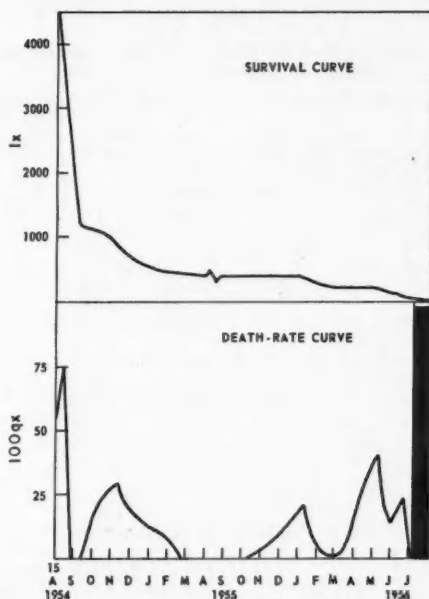


FIG. 1. Survival and death-rate curves; Mount Eisenhower, valley bottom plus 750 ft (5400 ft), 1954-56 generation.

indicated that they hibernate with no food in the gut, thereby increasing their cold resistance (32). No significant mortality was observed at temperatures maintained at  $-8^{\circ}\text{F}$  for 24 hours. A detailed discussion of the relation of climate to winter mortality is presented in a previous report (42). It was demonstrated, from a comparison of winter mortality estimates, that larval populations can have a high survival if January minimum temperatures of  $-30^{\circ}\text{F}$  to  $-40^{\circ}\text{F}$  do not persist long enough to depress the mean monthly temperature to the zero mark or if the weather is not abnormally severe in other winter months.

There appears to be little doubt that winter temperatures have been the main cause for the decline in needle miner populations. High mortality was first observed in 1946 (13) but populations were still high until the winter of 1949-50 (11). Since 1950 the population has been reduced (in numbers) and has been restricted to the intermediate levels on the mountain slopes where it was first noted. The percentage mortality for the whole generation is more significant when based on the first-instar larval population than those based on yearly estimates of the population present before hibernation (Table IX). A detailed discussion of year-to-year variations of winter mortality has been presented elsewhere (42).

The importance of winter mortality is clearly shown when compared with other control factors (Fig. 9). Estimates of average parasitism for comparable

TABLE II  
Life table for the 1956-1958 generation of needle miner,  
Mount Eisenhower (5400-ft elevation)

| <i>X</i>  | <i>lx</i>  | <i>dxF</i>   | <i>dx</i>                | 100 <i>qx</i>        |
|---|------------|--|--------------------------|----------------------|
| <i>X</i> <sub>1</sub> : eggs, July, 1956  | 378        | Needle drop<br>Resination  | 61<br>9                  | 16.14<br>2.38        |
|   |            |  | 70                       | 18.52                |
| <i>X</i> <sub>2</sub> : I and II instars, Sept., 1956                             | 308        | Winter mortality<br>Spring mortality<br>Out-of-phase larvae                | 36<br>0<br>11            | 11.69<br>—<br>3.57   |
|   |            |  | 47                       | 15.26                |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars,<br>July, 1957 | 261        | Winter mortality<br>Spring mortality<br>Others including bird<br>predation | 14<br>11<br>2            | 5.36<br>4.21<br>0.77 |
|   |            |  | 27                       | 10.34                |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1958                               | 234        | Larval parasitism  | 95                       | 40.59                |
| <i>X</i> <sub>6</sub> : Pupae, June, 1958   | 139        | Pupal parasitism<br>Unknown pupal mortality<br>Moths trapped in needles    | 0<br>8<br>3              | —<br>5.76<br>2.16    |
|   |            |  | 11                       | 7.92                 |
| Emergents   | 128        |  |                          |                      |
| Sex ratio 47:53   | 60F 68M    |  |                          |                      |
| Generation mortality  |            |  | 250                      | 66.14                |
| Expected number of eggs   | = 720-2220 |  |                          |                      |
| Measured number   | = 328      |  | Population trend = 86.8% |                      |
| Number of larvae established  | = 215      |  | Population trend = 69.8% |                      |

areas were divided between the two generation years to make them comparable to winter mortality, for which yearly estimates are available, i.e. they are additive. All other mortality factors, excluding those acting on the egg population, are combined; data on these factors are available from 1955 to 1958. For the years measured it would appear that parasitism was controlled in a manner similar to population, at least up to the 1956-58 generation.

## (2) Spring Mortality

A small percentage of larvae killed after feeding each spring has been found since detailed sampling for life table studies was commenced in 1954. This mortality never exceeded 8% of the larval population entering hibernation. It has long been recognized that spring mortality can be an important factor, particularly in open-feeding insects (4, 12, 52). Early studies on the needle miner indicate that the commencement of feeding in the spring is largely dependent on spring temperatures. Generally it appears that feeding ceases

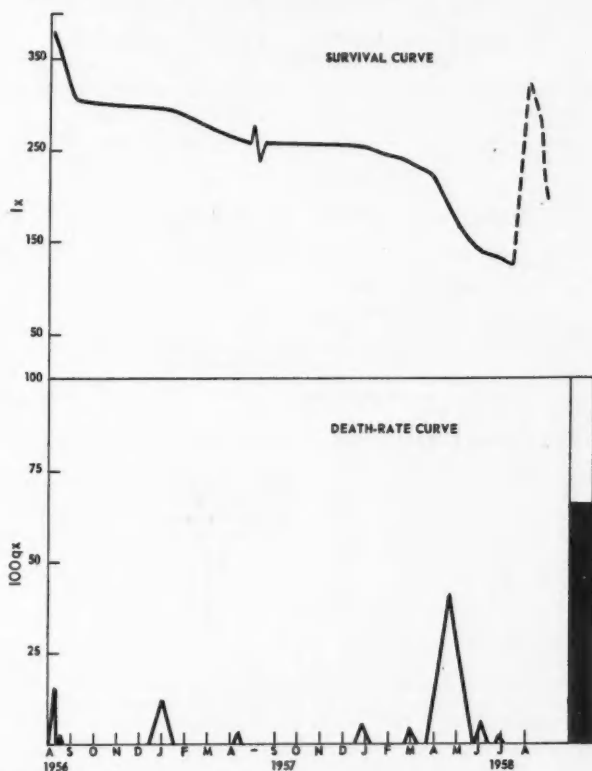


FIG. 2. Survival and death-rate curves; Mount Eisenhower, valley bottom plus 750 ft (5400 ft); 1956-58 generation.

in the fall when maximum temperatures fall below 45° F and the minimum temperatures are commonly below freezing. The reverse of these conditions is associated with commencement of spring feeding (37). Spring frosts are common and may be severe enough to cause the mortality observed.

### (3) Possible Mortality from Other Climatic Factors

#### (a) Eggs and First-instar Larvae

No mortality of eggs in the field or laboratory has been observed. They are apparently able to endure any field condition which occurred in the 3 years that egg sampling was carried out. However, large reductions in populations between oviposition and larval establishment may occur periodically. This was shown in 1954 when a count of 4700 eggs per 100 tips was made and establishment was only 1114 larvae per 100 tips.

Two sources of loss have been observed in the field but techniques have not been designed by which they can be evaluated except by subtraction from

TABLE III

Life table for the 1954-1956 generation of needle miner,  
Massive Range (5500-ft elevation)

| <i>X</i>  | <i>lx</i>                | <i>dxF</i>               | <i>dx</i> | 100 <i>qx</i> |
|---|--------------------------|--------------------------|-----------|---------------|
| <i>X</i> <sub>1</sub> : eggs  | Not measured             |                          |           |               |
| <i>X</i> <sub>2</sub> : I and II instars, Sept., 1954                             | 1257                     | Winter mortality         | 737       | 58.60         |
|   |                          | Spring mortality         | 55        | 4.38          |
|   |                          | Out-of-phase larvae      | 0         | —             |
|   |                          |                          | 792       | 62.98         |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars,<br>July, 1955 | 465                      | Winter mortality         | 94        | 20.22         |
|   |                          | Spring mortality         | 2         | 0.43          |
|   |                          | Bird predation           | 124       | 26.67         |
|   |                          |                          | 220       | 47.32         |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1956                               | 245                      | Larval parasitism        | 123       | 50.20         |
| <i>X</i> <sub>6</sub> : Pupae, June, 1956   | 122                      | Pupal parasitism         | 0         |               |
|   |                          | Unknown pupal mortality  | 30        | 24.84         |
|   |                          | Moths trapped in needles | 0         |               |
|   |                          |                          | 30        | 24.84         |
| Emergents   | 92                       |                          |           |               |
| Sex ratio 55:45   | 51F 41M                  |                          |           |               |
| Generation mortality  |                          |                          | 1165      | 92.68         |
| Expected number of eggs   | = 492-1517               |                          |           |               |
| Measured number—inadequate sample   |                          |                          |           |               |
| Number of larvae established  | = 190                    |                          |           |               |
|   | Population trend—unknown |                          |           |               |
|   | Population trend = 15.1% |                          |           |               |

present sampling stages. One of these is the drop of mined needles containing eggs, and the other is the prevention of larval establishment by adverse weather factors. Examination of the needle litter at the base of trees has substantiated that drop of mined needles containing eggs does occur occasionally. Morgan (25) estimated this loss to be 18% in the California needle miner in 1955. Since loss of eggs did not occur in the Bow Valley in 1956 or 1958, it is probable that this form of loss is intermittent, perhaps resulting from strong gusts of wind during the time of egg development. No measurement has been made of the loss of first-instar larvae but it is recognized that it could be an important factor in population reduction. Experiments by Shepherd (34) indicated that the threshold of activity of newly emerged larvae is rather high (59-65° F), and limited experiments indicated that high humidities restrict first-instar larval activity. Examination of hygrothermograph records in 1954 indicate a cold, wet period from August 18 to 24 which corresponds closely to the hatching period. Again, no measurement of loss is possible except by subtraction in the life table but it is reasonably certain that the combination of low temperature and high humidity is at least partly responsible for the loss noted in 1954 between oviposition and larval establishment. This loss did not

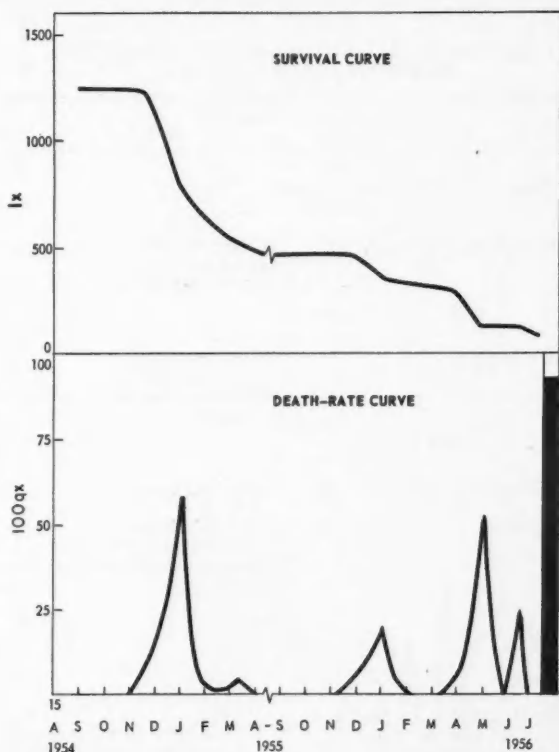


FIG. 3. Survival and death-rate curves; Massive Mountain, valley bottom plus 600 ft (5500 ft); 1954-56 generation.

occur in 1956, in which year conditions were more favorable for larval establishment (41).

(b) *Larvae during the Summer*

The suggestion by Morgan (25) that needle miner larvae are forced from their mines by excessive heat has not been observed under Canadian conditions. Although mortality has not been found during summer when only larvae are present, adverse climatic factors during this period may have a long-term effect on the population by influencing larval development. For example, the cool, wet summer of 1951 in Yoho Park extended the normal time for larval transfer from first to second needles by 2 to 3 weeks. Such a delay in larval transfer could reduce populations by its effects on winter survival and possibly on fecundity.

(c) *Pupae*

The mortality of pupae in all areas except Mount Cathedral in Yoho Park was estimated at from 20 to 25% in 1956 and as high as 40% in 1958. The cause of pupal mortality is unknown but is believed to be of climatic origin.

TABLE IV  
Life table for the 1956-1958 generation of needle miner,  
Massive Range (5500-ft elevation)

| <i>X</i>   | <i>lx</i>         | <i>dxF</i>                | <i>dx</i> | 100 <i>qx</i> |
|--|-------------------|---------------------------|-----------|---------------|
| <i>X</i> <sub>1</sub> : Eggs, July, 1956                                       | Inadequate sample |                           |           |               |
| <i>X</i> <sub>2</sub> : I and II instars, Sept., 1956                          | 190               | Winter mortality          | 30        | 15.79         |
|  |                   | Spring mortality          | 6         | 3.16          |
|  |                   | Out-of-phase larvae       | 3         | 1.58          |
|  |                   |                           | 39        | 20.53         |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars, July, 1957 | 151               | Winter mortality          | 23        | 15.23         |
|  |                   | Spring mortality          | 13        | 8.61          |
|  |                   | Bird predation            | 1         | 0.66          |
|  |                   | Unknown                   | 52        | 34.44         |
|  |                   |                           | 89        | 58.94         |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1958                            | 62                | Larval parasitism         | 29        | 46.77         |
| <i>X</i> <sub>6</sub> : Pupae, June 1958                                       | 33                | Pupal parasitism          | 0         |               |
|  |                   | Unknown pupal mortality   | 10        | 30.30         |
|  |                   | Moths trapped in needles  | 3         | 9.09          |
|  |                   |                           | 13        | 39.39         |
| Emergents  | 20                |                           |           |               |
| Sex ratio 58:42  | 12F 8M            |                           |           |               |
| Generation mortality   |                   |                           | 170       | 89.47         |
| Expected number of eggs = 96-296   |                   |                           |           |               |
| Measured number—inadequate sample  |                   | Population trend—unknown  |           |               |
| Number of larvae established = 195   |                   | Population trend = 102.2% |           |               |

#### (d) Adults

The separate activities of flight and oviposition, as well as fecundity and fertility, were considered together in relation to abundance of adults. Detailed information on this stage is available for only three generations: 1954, 1956, and 1958. While estimates of potential population are admittedly crude, some workers have shown by dissection that minimum egg potential approaches 100 eggs per female (unpublished data) but for the present purposes 12 to 37 eggs are used. The choice of this range was based partly on the results of recent moth dissections and egg-sampling results. Fecundity may be adversely affected by conditions during the developmental period (33, 52) and field samples indicate that fewer eggs are laid than is usually expected. No abnormalities of ovaries were observed in moths.

Pairing of captive adults was largely unsuccessful, but for those females that did lay eggs the range was from 1 to 19, the average only 8 eggs per female. Field sampling, where successful, indicated an average of about 8 eggs per female also. Field observations and population sampling indicated that the number of eggs laid, at least in the last two moth flights, is lower

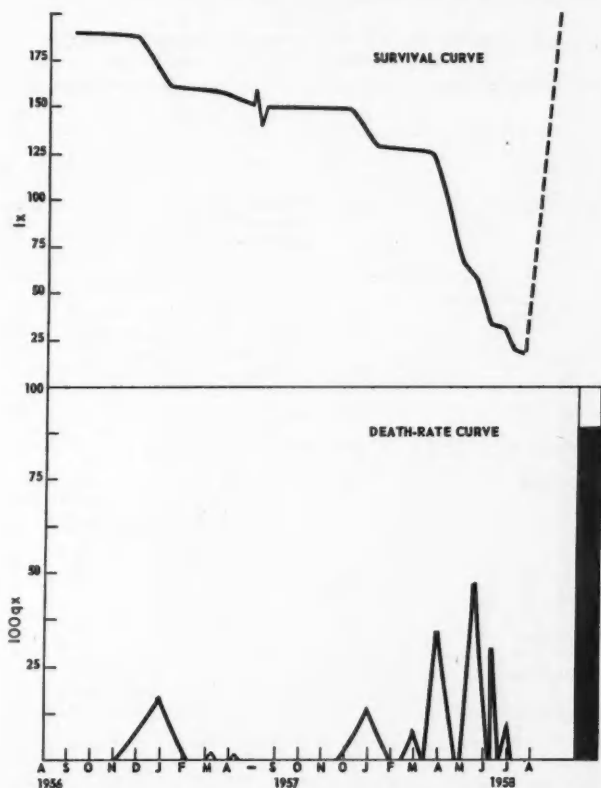


FIG. 4. Survival and death-rate curves; Massive Mountain, valley bottom plus 600 ft (5500 ft); 1956-58 generation.

than the bottom of the estimated range. Oviposition is very easily disrupted and this could be a very significant factor in population reduction, depending largely on the weather during the egg-laying period. Possibly greater inhibition of oviposition in 1956 and 1958 than in 1954 was indicated by egg samples, since fewer large egg masses were found in these later years than in 1954 and the number of single eggs found in proportion to the total number was far greater.

Cold, windy, or rainy weather has an important effect on moth flight and oviposition. Field observations in 1956 and 1958 indicated that moths did not lay at temperatures below  $45^{\circ}\text{F}$  and at wind speeds above 3 to 5 m.p.h. Another less obvious effect was observed in 1958 when a very severe storm, accompanied by high winds and heavy rain, occurred during the moth flight between the emergence times of males and females. Normally males emerge first, the proportion of males to females becoming about equal approximately

TABLE V  
Life table for the 1954-1956 generation of needle miner,  
Mount Girouard (6000-ft elevation)

| <i>X</i>  | <i>lx</i> | <i>dxF</i>  | <i>dx</i>             | 100 <i>qx</i>                 |
|---|-----------|---|-----------------------|-------------------------------|
| <i>X</i> <sub>1</sub> : eggs, not measured  |           |   |                       |                               |
| <i>X</i> <sub>2</sub> : I and II instar, Sept., 1954                              | 2633      | Winter mortality<br>Spring mortality<br>Out-of-phase larvae             | 1593<br>144<br>0      | 60.50<br>5.46                 |
|   |           |   | 1737                  | 65.96                         |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars,<br>July, 1955 | 896       | Winter mortality<br>Spring mortality<br>Bird predation<br>Unknown       | 213<br>15<br>76<br>41 | 23.77<br>1.67<br>8.50<br>4.60 |
|   |           |   | 345                   | 38.54                         |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1956                               | 551       | Larval parasitism   | 288                   | 52.27                         |
| <i>X</i> <sub>6</sub> : Pupae, June, 1956   | 263       | Pupal parasitism<br>Unknown pupal mortality<br>Moths trapped in needles | 2<br>56<br>0          | 7.60<br>21.29                 |
|   |           |   | 58                    | 28.89                         |
| Emergents   | 205       |   |                       |                               |
| Sex ratio 53:47   | 109F 96M  |   |                       |                               |
| Generation mortality  |           |   | 2428                  | 92.21                         |
| Expected number of eggs = 1152-3552   |           |   |                       |                               |
| Measured number = 385   |           | Population trend—unknown  |                       |                               |
| Number of larvae established = 395  |           | Population trend = 14.6%  |                       |                               |

2 weeks after the beginning of emergence. Such a storm, occurring when a preponderance of males was present, could have reduced the male population to a point where many of the females would not be mated. Factors such as outlined above could explain the continued decline in population even though winter mortality, parasitism, and other factors were low (Fig. 9).

The moths are crepuscular. Diminution of light (or radiation), such as occurs during cloudy periods, caused increased activity during the day. Peak flight activity is at sunset, provided there is no wind or rain, the moths flying upwards to the tops of the tree crowns, giving rise to the distribution of eggs and larvae found (38). While fluctuations in barometric pressure caused increased flight activity in some insects (44, 53) this did not appear to be a factor influencing needle miner flight activity.

#### PARASITISM

This paper considers only the total role of parasites in the dynamics of the past outbreak of the lodgepole needle miner. Information on parasite biologies and dynamics and the breakdown of the parasite complex by species in supplementary life tables is planned for a separate publication.

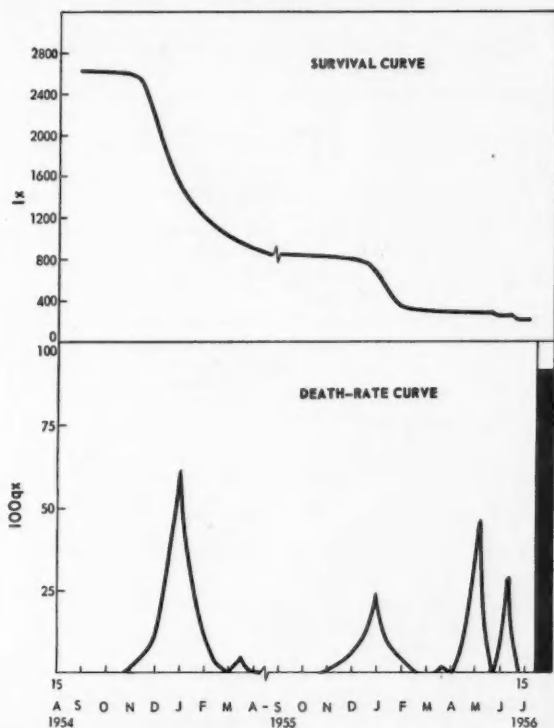


FIG. 5. Survival and death-rate curves; Mount Girouard, valley bottom plus 700 ft (6000 ft); 1954-56 generation.

Parasites have long been upheld as the major influence in so-called "biotic" control and many instances have been cited where economic control has been effected, largely through the introduction of parasites. Sweetman (45) subjected many stated examples to intense scrutiny and concluded that many of these were attributable to factors other than biotic. Andrewartha and Birch (2) and Milne (24) point out that there are few or no proved cases of control of an insect population by its parasites and that abundant evidence exists that they are unable to do so. The current study recognizes the possibility of control through parasites, at least to the extent of gaining an understanding of them as they affect the dynamics of needle miner populations.

It has been shown (Fig. 9) that parasitism was not as important a factor as winter mortality when compared with total population, for any generation except 1956-58, and did not play an important part in reducing needle miner populations. In fact, parasitism appeared to be controlled by climate in the same manner as was the needle miner population. However, parasitism achieves considerable importance in the later part of the life cycle when its effect on the population surviving the two winters is considered (Table X).

TABLE VI  
Life table for the 1956-1958 generation of needle miner,  
Mount Girouard (6000-ft elevation)

| <i>X</i>  | <i>lx</i> | <i>dxF</i>  | <i>dx</i>       | 100 <i>qx</i>         |
|---|-----------|---|-----------------|-----------------------|
| <i>X</i> <sub>1</sub> : eggs, July, 1956  | 385       | Needle drop<br>Resination   | 0<br>0          |                       |
| <i>X</i> <sub>2</sub> : I and II instar, Sept., 1956                              | 395       | Winter mortality<br>Spring mortality<br>Out-of-phase larvae                     | 126<br>18<br>32 | 31.90<br>4.56<br>8.10 |
|   |           |   | 176             | 44.56                 |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars,<br>July, 1957 | 219       | Winter mortality<br>Spring mortality<br>Bird predation and<br>unknown mortality | 15<br>0<br>< 1  | 6.85<br><br>< .01     |
|   |           |   | 15+             | 6.85                  |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1958                               | 204       | Larval parasitism   | 111             | 54.41                 |
| <i>X</i> <sub>6</sub> : Pupae, June, 1958   | 93        | Pupal parasitism<br>Unknown pupal mortality<br>Moths trapped in needles         | 0<br>38<br>6    | <br>40.86<br>6.45     |
|   |           |   | 44              | 47.31                 |
| Emergents   | 49        |   |                 |                       |
| Sex ratio 46:54   | 23F 26M   |   |                 |                       |
| Generation mortality  |           |   | 346             | 87.59                 |
| Expected number of eggs = 276-851   |           |   |                 |                       |
| Measured number = 327   |           | Population trend = 84.9%  |                 |                       |
| Number of larvae established = 334  |           | Population trend = 84.6%  |                 |                       |

Estimates of parasitism are probably low as there is no way of determining mortality from parasitism in the early stages of the host except by internal examination of needle miner larvae. Practical considerations do not permit such sampling at present. When the actual numbers of parasites are compared on the same basis as for the host (number per branch tip) it is evident that the actual number of parasites is declining but at a slower rate than the host (Fig. 9). The possibility exists that the parasite complex of the lodgepole needle miner, *Recurvaria starki* Free., has been kept at low levels of abundance during the past needle miner outbreak by a combination of factors which permitted host increase but inhibited parasite success.

#### PREDATION

No significant loss in eggs or larvae could be attributed to predation at any time prior to 1956. Predation of eggs has not been observed and only limited and localized predation of larvae by birds is believed to occur. Numerous needles that had been shredded, presumably by birds, were noted in two areas in 1956, Mt. Girouard and Massive, but predation was again negligible in

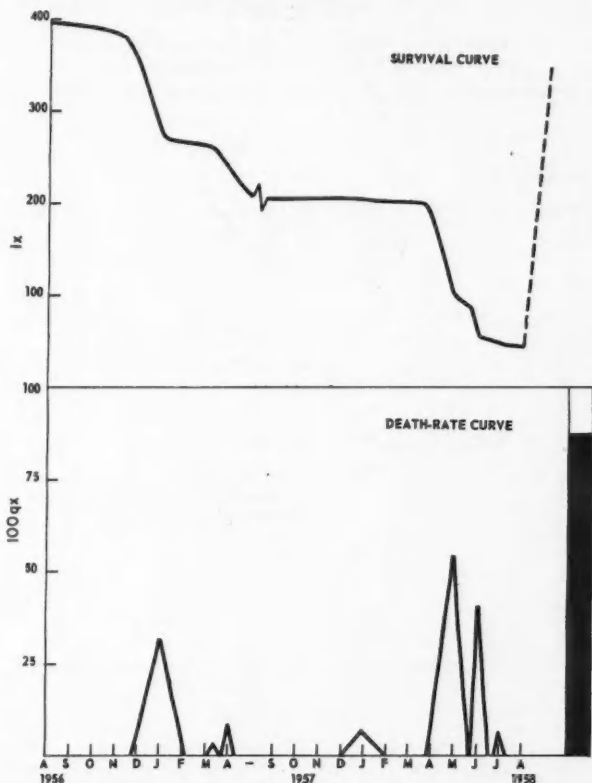


FIG. 6. Survival and death-rate curves; Mount Girouard, valley bottom plus 700 ft (6000 ft); 1956-58 generation.

1958. The most probable predators were chickadees, Rocky Mountain jays, and juncos. The importance of predation in the dynamics of needle miner populations, while difficult to assess, is believed to be low. On the other hand, predation could assume greater importance at times when needle miner populations are low and restricted to distinct refuge areas.

#### DISEASE

Disease has not been an important factor in the needle miner outbreak. The occurrence of diseased larvae reported in 1945 and 1946 (13) is now in doubt; winter-killed larvae were mistaken for diseased ones (Hopping, G.R., personal communication). Disease was not apparent before 1952 and since that time only a few diseased larvae have been detected. Adults have been similarly disease-free. A virus disease was isolated from the California needle miner in 1952 but attempts to introduce this into the Canadian population failed (unpublished data).

TABLE VII  
Life table for the 1954-1956 generation of needle miner,  
Cathedral Mountain (4700-ft elevation)

| <i>X</i>  | <i>lx</i> | <i>dxF</i>                 | <i>dx</i> | 100 <i>qx</i> |
|---|-----------|----------------------------|-----------|---------------|
| <i>X</i> <sub>1</sub> : eggs, not measured  |           |                            |           |               |
| <i>X</i> <sub>2</sub> : I and II instars, Sept., 1954                             | 924       | Winter mortality           | 687       | 74.40         |
|   |           | Spring mortality           | 56        | 6.02          |
|   |           | Out-of-phase larvae        | 0         | —             |
|   |           |                            | 743       | 80.42         |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars,<br>July, 1955 | 181       | Winter mortality           | 111       | 61.33         |
|   |           | Spring mortality           | 6         | 3.31          |
|   |           | Bird predation and unknown | 0         | —             |
|   |           |                            | 117       | 64.64         |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1956                               | 64        | Larval parasitism          | 22        | 34.38         |
| <i>X</i> <sub>6</sub> : Pupae, June, 1956   | 42        | Pupal parasitism           | 0         | —             |
|   |           | Unknown pupal mortality    | 0         | —             |
|   |           | Moths trapped in needles   | 0         | —             |
| Emergents   | 42        |                            |           |               |
| Sex ratio: 49:51  | 21F 21M   |                            |           |               |
| Generation mortality  |           |                            | 882       | 95.45         |
| Expected number of eggs = 252-777   |           |                            |           |               |
| Measured number—inadequate sample   |           | Population trend—unknown   |           |               |
| Number of larvae established = 66   |           | Population trend = 7.1%    |           |               |

#### OTHER NATURAL CONTROL FACTORS

##### (1) *Resination*

Behavior of first-instar larvae of *Recurvaria starki* Free. and *R. milleri* Busck is almost identical. Upon eclosion they seek a green needle and begin to mine, almost invariably on the convex or outer surface of the needle (31, 39). The few that attempt to enter the needle from the concave or inner surface are usually killed. The abortive mine frequently has a small bubble of resin above it with the remains of the larva imbedded in the resin. The larval loss in the Canadian population has been negligible. Morgan (25) found similar mortality in California and estimated that 0.1% of the larval population died this way.

##### (2) *Competition and Overpopulation Factors*

There is no evidence to suggest that any other organism competes with the lodgepole needle miner for its food. The needle miner populations in the Canadian outbreak never reached the levels which would cause a critical food shortage. Intensive defoliation analyses that were begun in 1949 showed that populations did not persist at high levels long enough to cause complete defoliation. However, populations from 1940 to 1948 were high enough to cause about 80% defoliation in some localities and had these populations per-

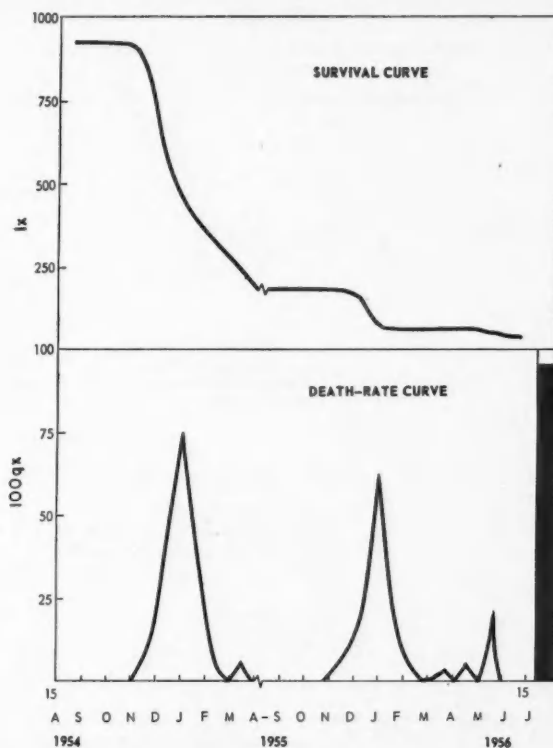


FIG. 7. Survival and death-rate curves; Cathedral Mountain, valley bottom plus 250 ft (4700 ft); 1954-56 generation.

sisted, food shortages would have occurred in localized areas (43). In this outbreak, therefore, competition is discounted as a significant population reduction factor.

"Overpopulation" factors (7, 35) have been credited by various authors with causing fluctuations in forest insect pests. However, the closely allied California needle miner has repeatedly reached the saturation point of its environment, killing forests over large areas without noticeable reduction in population prior to their own starvation and death. Survivors from those populations which caused total defoliation have caused outbreaks in new locations (25, 31).

### Epidemiology

#### THE THEORY OF CLIMATIC RELEASE

The importance of climate in the epidemiology of insect outbreaks has been a subject of controversy for many years. Early theories place the emphasis on biotic factors but some authors recognized that weather may cause an

TABLE VIII

Life table for the 1956-1958 generation of needle miner,  
Cathedral Mountain (4700-ft elevation)

| <i>X</i>   | <i>lx</i>         | <i>dxF</i>               | <i>dx</i> | 100 <i>qx</i> |
|--|-------------------|--------------------------|-----------|---------------|
| <i>X</i> <sub>1</sub> : eggs, July, 1956                                       | Inadequate sample |                          |           |               |
| <i>X</i> <sub>2</sub> : I and II instars, Sept., 1956                          | 66                | Winter mortality         | 22        | 33.33         |
|  |                   | Spring mortality         | 0         |               |
|  |                   | Out-of-phase larvae      | 14        | 21.21         |
|  |                   |                          | 36        | 54.54         |
| <i>X</i> <sub>3</sub> , <i>X</i> <sub>4</sub> : III and IV instars, July, 1957 | 30                | Winter mortality         | 2         | 6.67          |
|  |                   | Spring mortality         | 2         | 6.67          |
|  |                   | Bird predation           | <1        | 0.73          |
|  |                   | Unknown                  | <1        | 2.60          |
|  |                   |                          | 5         | 16.67         |
| <i>X</i> <sub>5</sub> : IV and V instars, May, 1958                            | 25                | Larval parasitism        | 10        | 40.00         |
| <i>X</i> <sub>6</sub> : Pupae, June, 1958                                      | 15                | Pupal parasitism         | 0         |               |
|  |                   | Unknown pupal mortality  | 3         | 20.00         |
|  |                   | Moths trapped in needles | 1         | 6.69          |
|  |                   |                          | 4         | 26.69         |
| Emergents  | 11                |                          |           |               |
| Sex ratio 60:40  | 7F 4M             |                          |           |               |
| Generation mortality   |                   |                          | 55        | 83.33         |
| Expected number of eggs  | = 48-148          |                          |           |               |
| Measured number  | = 35              | Population trend—unknown |           |               |
| Number of larvae established   | = 36              | Population trend = 54.6% |           |               |

"unbalance" which may lead to outbreaks (35). Later theories were more comprehensive (33, 36). Nicholson (27, 28, 29) believes that populations are in a state of balance and the main controlling factors (of numbers) are "density-dependent", a condition which includes direct competition for resources or space and parasites, predators, and pathogens. He claims that climate is "density-independent" and can never control populations. Andrewartha and Birch (2) hold that the factors of environment that control insect populations are numerous but that climatic factors are of major importance. They conclude that all factors are "density-dependent" and attach no special importance to biotic factors. Thompson (48, 49, 50) believes that natural control results from an organism living in a continuously fluctuating environment. Under favorable conditions, numbers increase; under unfavorable conditions, numbers decrease. Never do numbers increase indefinitely and rarely if ever, decrease to extinction. Fluctuations in population numbers tend to be inversely correlated with the complexity of the "ecosystem", a view held by many authors (1, 2, 16). Milne (24) reviewed the theories mentioned above and proposes his own, which he describes as a "modification of Thompson's".

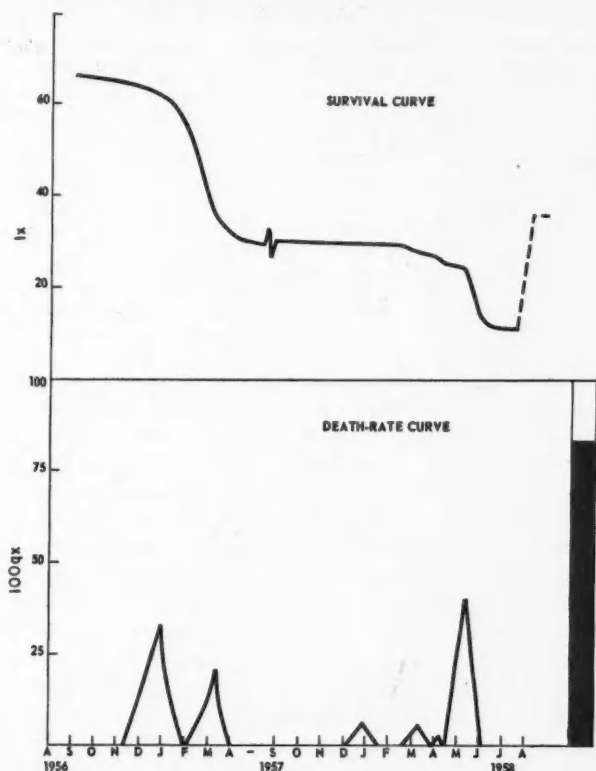


FIG. 8. Survival and death-rate curves; Cathedral Mountain, valley bottom plus 250 ft (4700 ft); 1956-58 generation.

He objects to the Nicholson theory on the grounds that competing species, parasites, predators, and pathogens can not control because they are "imperfectly density-dependent" and to Thompson's theory that it underestimates the importance of density-dependence. He also claims that the Andrewartha and Birch theory suffers from their treatment of density-dependence. Milne's own theory is that competition between individuals of a single species is "the only perfectly density-dependent factor" in nature. This factor is seldom evoked and therefore the control of increase is the combined action of factors, density-independent and "imperfectly" density-dependent. The control of decrease of numbers is brought about by density-independent factors.

Ulyett (51) has called climate a "catastrophic" factor and thinks it can be a contributory cause to insect outbreaks. This is based on the assumption that "density-dependent" (biotic) factors are more adversely affected by such catastrophes than the insect in question. In the absence of these controlling factors the insect may reach destructive densities when the catastrophe is

TABLE IX

Winter mortality of lodgepole needle miner: per cent mortality of whole generation based on number of established first-instar larvae

| Area                          | Year    |         |         |         |         |
|-------------------------------|---------|---------|---------|---------|---------|
|                               | 1948-50 | 1950-52 | 1952-54 | 1954-56 | 1956-58 |
| Mt. Eisenhower, valley bottom | 85      | —       | 99      | 100     | —       |
| Mt. Eisenhower, 5400 ft       | 78      | 33      | 45      | 70      | 21      |
| Massive                       | —       | —       | —       | 73      | 45      |
| Girouard                      | —       | —       | —       | 86      | 60      |
| Cathedral                     | —       | —       | —       | 68      | 65      |

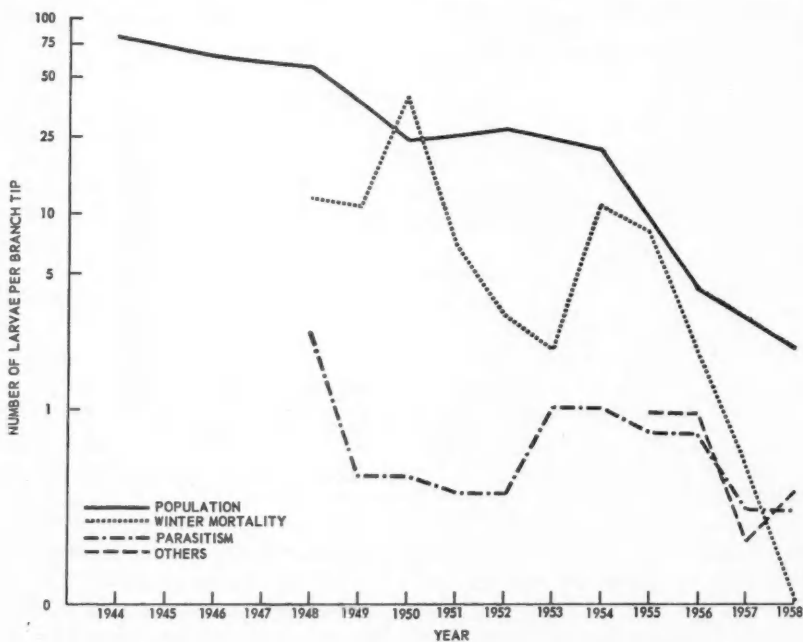


FIG. 9. Comparison of winter mortality, parasitism, and other mortality with respect to host population.

spent. Thalenhorst (46) has presented several European examples of insect outbreaks attributed to weather conditions. From observational evidence he shows that weather may influence an insect population in many ways: acting directly on the population, by its effect on some other factor which is fundamental to population growth (or lack of growth); by acting on the other factor and the population simultaneously with a reciprocal effect between population and factor; or by a maze of interactions involving soil, host-plant, population, and its enemies simultaneously, with interactions between the factors affected.

TABLE X  
Per cent parasitism of lodgepole needle miner

| Year | All averaged | Mt. Eisenhower, 5400 ft | Massive | Girouard | Cathedral |
|------|--------------|-------------------------|---------|----------|-----------|
| 1944 | 3            |                         |         |          |           |
| 1946 | 3            |                         |         |          |           |
| 1948 | 10           |                         |         |          |           |
| 1950 | 13           | 15                      | —       | 11       | —         |
| 1952 | 17           | 12                      | —       | —        | 22        |
| 1954 | 14           | 16                      | 10      | 16       | 14        |
| 1956 | 49           | 60                      | 50      | 52       | 34        |
| 1958 | 46           | 41                      | 47      | 54       | 40        |

The development of thought concerning weather in relation to insect outbreaks has slowly given more importance to weather as a causal agent. However, Wellington (56) has pointed out that although the literature is replete with papers dealing with the effects of various meteorological factors on many phases of insect development and behavior, only a few deal with those effects in terms of large scale weather processes and a very few follow through to the logical conclusion: prediction of biological phenomena with the aid of more modern methods of weather analysis and forecasting. Wellington (56) has developed probably the first inclusive theory relating insect abundance and weather which has been applied to three outbreak situations in Canada with considerable success (9, 55, 58):

"To assess climatic influences correctly it is necessary to examine climatic variations during the period immediately preceding or coinciding with the beginning of an outbreak of an insect that exhibits violent fluctuations in numbers instead of studying the climate while the outbreak exists. This follows from the concept of climatic release of a small indigenous population. That is, in a region where a species exists in small numbers, and in which biotic conditions already favour population growth no initial increase may occur until seasonal climatic control is relaxed. The important point to keep in mind however, is that favourable weather may have to recur several years in succession before a major increase in population can develop. Once the enormous potential for increase that such a species possesses is realized, the population grows so rapidly that no combination of adverse physical or biotic factors can halt it immediately. Since it is usually during this period that the outbreak is studied, it is not surprising that effects of the various original governing factors are often obscured."

In summary, the theory of climatic release explains the time and place of outbreaks and its worth may be measured by its ability to predict outbreaks. In its present stage of development, it does not explain the fluctuations in numbers in the manner of comprehensive theories. Studies on population dynamics in forest entomology during the periods of low numbers are rare,

although it is apparent that fluctuations in numbers without loss in "balance" are common and outbreaks the exception. Within the period of low numbers, increase in population from one year to the next can result from physical conditions becoming favorable to the insect. Readjustment of the population after this increase may come through density-related processes although these may not be entirely effective until physical conditions again become unfavorable. However, years with unfavorable weather conditions cannot always be expected to follow years with favorable conditions and eventually the favorable weather conditions occur several years in succession. During such a period, as the climatic theory postulates, the low populations may be released from the controlling influence of both physical and biotic factors (57).

#### THE THEORY OF CLIMATIC RELEASE APPLIED TO THE OUTBREAK OF THE LODGEPOLE NEEDLE MINER

A comparison of early weather records with those known to have caused a decline in needle miner populations showed that conditions which caused high mortality in needle miner populations occurred with relatively high frequency (Figs. 10 and 11). The longest interval between severe winters occurred from 1937 to 1950, the period during which the past needle miner outbreak occurred. The outbreak was discovered in 1942, confined to the middle altitudes. The fact that populations were found there and were increasing indicates that the outbreak was in a relatively early stage of development. Empirical calculations showed that the needle miner population could increase from a single pair per branch tip in 6 years (three generations) to numbers in excess of those found. If we assume one fertilized female per branch tip in the first year with an egg-laying capacity of eight eggs and a series of mild winters where total mortality did not exceed 20% in any one year, the population in the sixth year would be greater than 100 per tip. Thus, it is possible, beginning with the generation in 1938, that a comparable population growth to that postulated did occur owing to the series of mild winters following 1936-37. The assumption of one fertilized female per branch tip as an initial density is reasonable according to current samples (1958). The concentration of larvae per tip did not occur at the intermediate levels (elevations) probably because of dispersal throughout uninfested stands at other altitude levels and valley bottom (54). The severe winters commencing in 1945-46 undoubtedly reduced the populations to the lower densities of 1938 and earlier.

The needle miner outbreak studied was restricted to the valleys of Banff National Park and adjacent areas in Yoho and Kootenay parks. The failure of the needle miner to increase east of Banff Park may be explained on climatic grounds. The reasons for the failure to increase in the west are not so clear. The mean monthly temperatures at Edson, Rocky Mountain House, and Exshaw show that the eastern slopes of the Rocky Mountains are generally colder than the outbreak area. This is also demonstrated in the Climatological Atlas for Canada (47). This is because the cold cA air usually flows south across the prairies and then moves laterally into the outbreak area from an

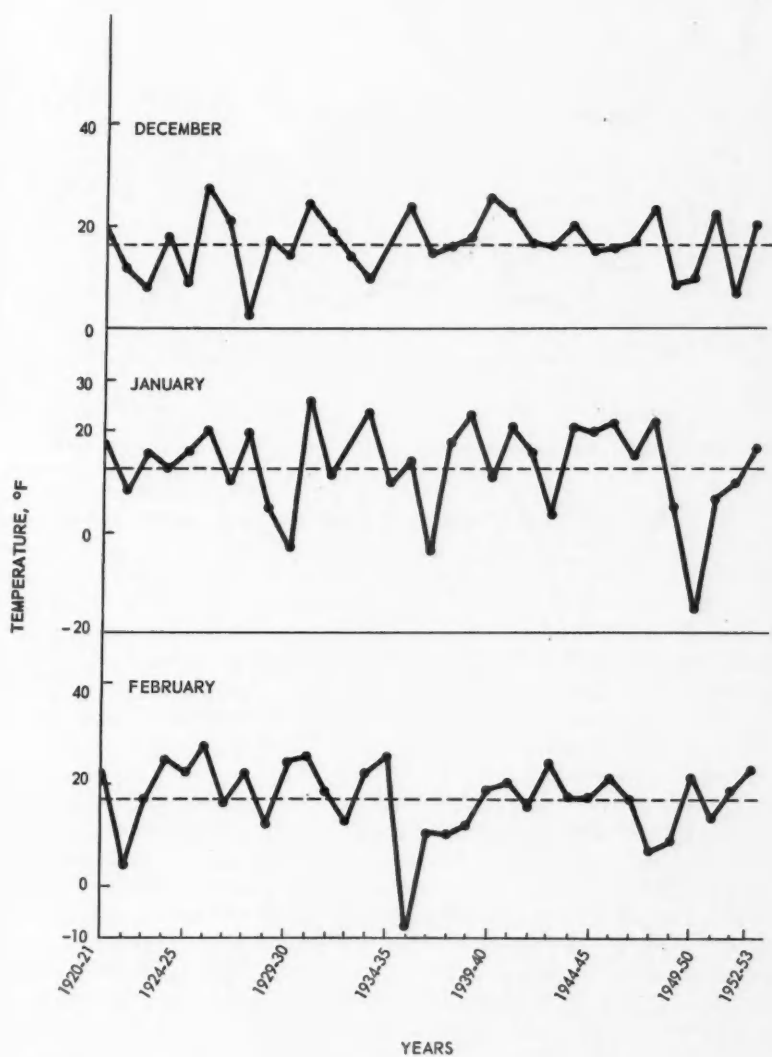


FIG. 10. Mean monthly temperatures 1920-21 to 1952-53 for Banff, Alberta.

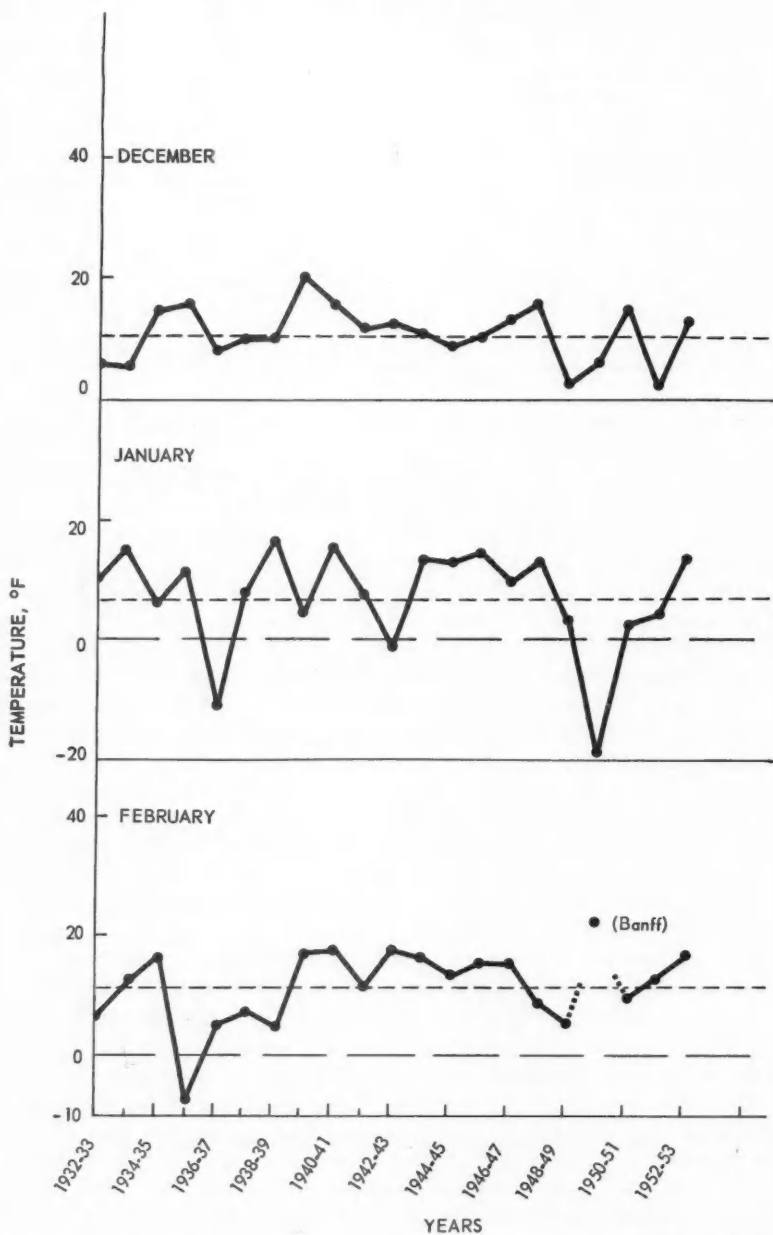


FIG. 11. Mean monthly temperatures 1932-33 to 1952-53 for Lake Louise, Alberta.

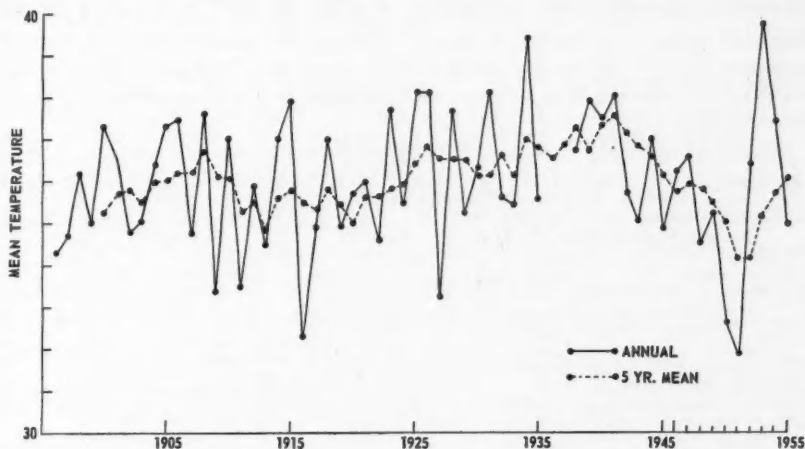


FIG. 12. Mean annual temperature and 5-year running average annual mean temperature for Banff, Alberta, 1896 to 1955.

easterly or northeasterly direction. The mountains frequently block or diminish this penetration into the valleys. Circulation in Yoho and Kootenay parks is more complex than that in Banff Park (11). Climatic fluctuations in these areas are often more violent than in Banff Park, even though the winter extremes may not be as severe. The failure of needle miner populations to increase west of Banff Park may be linked to violent fluctuations of climate acting upon stages other than larvae, rather than to sustained low temperatures, as in Banff Park.

Yearly average temperatures were calculated for Banff, Alberta, plotted as a 5-year running average (Fig. 12) (19). A definite warming period is shown from about 1925 to 1948. However, there were several years prior to 1936-37 when the winters were comparable to that of 1949-50; this supports the assumption that the outbreak began about 1938. Evidence of a real climatic change not attributable to random fluctuations has been compiled and it shows that the climate of northern regions of the world did become warmer about 1940 (56). From the weather records presented in the text and others available it is unlikely that an outbreak of comparable magnitude was able to occur prior to that time. Tree ring studies do not show any evidence of a previous outbreak (26, 43). It would follow from these observations that in this region the "normal" climate is too severe to permit the occurrence of sustained outbreaks of *Recurvaria starki* Free.

#### Acknowledgments

Grateful acknowledgments are due the following: K. Graham, University of British Columbia; W. R. Henson, Yale University, who permitted the use of portions of his air-mass analysis data and contributed to the theoretical

considerations; and G. R. Hopping, J. A. Cook, and R. F. Shepherd of the Forest Biology Laboratory, Calgary, Alberta. Thanks also are due the personnel of the Calgary and Edmonton meteorological offices of the Department of Transport, whose heads gave free access to their records and data.

### References

1. ALLEE, W. C., EMERSON, A. E., PARK, O., PARK, T., and SCHMIDT, K. P. Principles of animal ecology. W. B. Saunders Co., Philadelphia, Pa. 1949.
2. ANDREWARTHA, H. G. and BIRCH, L. C. The distribution and abundance of animals. Univ. Chicago Press, Chicago, Ill. 1954.
3. BATZER, H. O. and BENJAMIN, D. M. Cold temperature tolerance of the European pine shoot moth in lower Michigan. J. Econ. Entomol. **47**, 801-803 (1954).
4. BLAIS, J. R., PRENTICE, R. M., SIPPPELL, W. L., and WALLACE, D. R. Effects of weather on the forest tent caterpillar, *Malacosoma disstria* Hbn., in central Canada in the spring of 1953. Can. Entomologist, **87**, 1-8 (1955).
5. BROWN, A. W. A. In Ann. Rept. Forest Insect Survey. Can. Dept. Agr., Div. Entomol., Sci. Serv., Ottawa. 1942.
6. DEBACH, P., FISHER, T. W., and LANDI, J. Some effects of meteorological factors on all stages of *Aphytis lingnanensis*, a parasite of California red scale. Ecology, **36**, 743-753 (1955).
7. FRANZ, J. Über die genetischen Grundlagen des Zusammenbruchs einer Massenvermehrung aus inneren Ursachen. Z. angew. Entomol. **31**, 228-260 (1949).
8. GRAHAM, K. and STARK, R. W. Lodgepole needle miner (bionomics). Can. Dept. Agr., Div. Entomol. Bi-Monthly Progr. Rept. **5**(6), 2 (1949).
9. GREENBANK, D. O. The role of climate and dispersal in the initiation of outbreaks of the spruce budworm. I. The role of climate. Can. J. Zool. **34**, 453-476 (1956).
10. HARVILLE, J. P. Ecology and population dynamics of the California oak moth *Phryganidia californica* Packard (Lepidoptera: Dioptidae). Ph.D. Thesis, Stanford University, California. 1956.
11. HENSON, W. R., STARK, R. W., and WELLINGTON, W. G. Effects of the weather of the coldest month on winter mortality of the lodgepole needle miner, *Recurvaria* sp. in Banff National Park. Can. Entomologist, **86**, 13-19 (1954).
12. HODSON, A. C. An ecological study of the forest tent caterpillar, *Malacosoma disstria*. Hbn. in northern Minnesota. Univ. Minn. Agr. Expt. Sta. Tech. Bull. **148** (1941).
13. HOPPING, G. R. Lodgepole pine needle miner. Can. Dept. Agr., Div. Entomol. Bi-Monthly Progr. Rept. **2**(4), 3 (1946).
14. HOPPING, G. R. The lodgepole pine needle miner in western Canadian parks. Can. Dept. Agr., Div. Entomol. Proc. Publ. **59**. Ottawa. 1946.
15. HOPPING, G. R. Rocky Mountain region. Can. Dept. Agr., Div. Entomol. Bi-Monthly Progr. Rept. **4**(4), 3 (1948).
16. HOPPING, G. R. Timber types in relation to insect outbreaks in the Canadian Rocky Mountains. 81st Ann. Rept. Entomol. Soc. Ontario, 72-75 (1950).
17. HUTCHINSON, R. N. Influence of winter night temperatures on the California red scale. J. Econ. Entomol. **40**, 921-922 (1947).
18. LEECH, H. B. In Ann. Rept. Forest Insect Survey—British Columbia and western Alberta. Can. Dept. Agr., Div. Entomol., Sci. Serv., Ottawa. 1944.
19. LONGLEV, R. W. Mean annual temperatures and running mean temperatures for selected Canadian stations. Can. Dept. Transport, Meteorol. Div., Circ. **2481**. Tec. **186**. 1954.
20. LORD, F. T. and MCPHEE, A. W. The influence of spray programs on the fauna of apple orchards in Nova Scotia. VI. Low temperatures and the natural control of the oystershell scale, *Lepidosaphes ulmi* (L.) (Homoptera: Coccidae). Can. Entomologist, **85**, 282-291 (1953).
21. MCGUFFIN, W. C. In Annual report of the Forest Insect Survey. Can. Dept. Agr., Div. Entomol., Ottawa. 1948.
22. MCGUFFIN, W. C. Forest Insect Survey. Can. Dept. Agr., Div. Entomol. Bi-Monthly Progr. Rept. **5**(2), 3-4 (1949).
23. MILLS, H. B. Weather and climate. In U.S.D.A. yearbook, Insects. 1952.
24. MILNE, A. The natural control of insect populations. Can. Entomologist, **89**, 193-213 (1957).
25. MORGAN, F. D. Factors influencing the abundance of *Recurvaria milleri* Busck (Lepidoptera: Gelechiidae). Ph.D. Thesis, University of California, Berkeley, California. 1955.
26. MOTT, D. G., NAIRN, L. D., and COOK, J. A. Radial growth in forest trees and effects of insect defoliation. Forest Sci. **3**, 286-304 (1957).
27. NICHOLSON, A. J. The balance of animal populations. J. Anim. Ecol. **2**, 132-178 (1933).

28. NICHOLSON, A. J. Fluctuation of animal populations. Rept. Australian New Zealand Assoc. Advance. Sci. **26**, 134-148 (1947).
29. NICHOLSON, A. J. An outline of the dynamics of animal populations. Australian J. Zool. **2**, 9-65 (1955).
30. NOLTE, H. W. Beitrage zur Epidemiologie und Prognose des Rapserrdflohs (*Psylliodes chrysocephala* L.). Beitr. Entomol. (Berlin) **3**, 518-529 (1953).
31. PATTERSON, J. E. Life history of *Recurvaria milleri* Busck, the lodgepole needle miner in Yosemite National Park, California. J. Agr. Research, **21**, 127-142 (1921).
32. SALT, R. W. Influence of moisture content and temperature on cold-hardiness of hibernating insects. Can. J. Zool. **34**, 283-294 (1956).
33. SCHWERDTFEGER, F. Grundriss der Forstpathologie. Paul Parey, Berlin. 1950.
34. SHEPHERD, R. F. Lodgepole needle miner, *Recurvaria milleri* Busck. Can. Dept. Agr., Div. Entomol. Bi-Monthly Progr. Rept. **6**(6), 3 (1950).
35. SOLOMON, M. E. The natural control of animal populations. J. Animal Ecol. **18**, 1-35 (1949).
36. SOLOMON, M. E. Dynamics of insect populations. In Ann. Rev. Entomol. **2**, 121-142 (1957). Palo Alto, Calif.
37. STARK, R. W. Lodgepole needle miner. Can. Dept. Agr., Div. Entomol. Bi-Monthly Progr. Rept. **5**(1), 3 (1949).
38. STARK, R. W. Analysis of a population sampling method for the lodgepole needle miner in Canadian Rocky Mountain Parks. Can. Entomologist, **84**, 316-321 (1952).
39. STARK, R. W. Distribution and life history of the lodgepole needle miner (*Recurvaria* sp.) (Lepidoptera: Gelechiidae) in Canadian Rocky Mountain Parks. Can. Entomologist, **86**, 1-12 (1954).
40. STARK, R. W. Life tables for the lodgepole needle miner, *Recurvaria starki* Free. (Lepidoptera: Gelechiidae). Proc. Xth Intern. Congr. Entomol. **4**, 151-162 (1958).
41. STARK, R. W. Population dynamics of the lodgepole needle miner, *Recurvaria starki* Free. (Lepidoptera: Gelechiidae) in Canadian Rocky Mountain Parks. Ph.D. Thesis, University of British Columbia, Vancouver, B.C. 1958.
42. STARK, R. W. Climate in relation to winter mortality of the lodgepole needle miner, *Recurvaria starki* Free. in Canadian Rocky Mountain Parks. Can. J. Zool. **37**, 753-761 (1959).
43. STARK, R. W. and COOK, J. A. The effects of defoliation by the lodgepole needle miner (*Recurvaria starki* Free.). Forest Sci. **3**, 376-396 (1957).
44. STEPHEN, W. P. and BIRD, R. D. The effect of barometric pressure upon oviposition of the imported cabbageworm, *Pieris rapae* (L.). Can. Entomologist, **81**, 132 (1949).
45. SWEETMAN, H. L. Successful examples of biological control of pest insects and plants. Bull. Entomol. Research, **26**, 373-377 (1935).
46. THALENHORST, W. Other aspects of population dynamics (in German). Twelfth Congress IUFRO 56/24/19. 1956.
47. THOMAS, M. K. Climatological atlas for Canada. Dept. Transport, Meteorol. Div., Div. Building Research, N.R.C. No. 3151(41), Ottawa. 1953.
48. THOMPSON, W. R. On natural control. Parasitology, **21**, 269-281 (1929).
49. THOMPSON, W. R. Biological control and the theories of the interactions of populations. Parasitology, **31**, 299-388 (1939).
50. THOMPSON, W. R. Theory of natural and biological control. In Ann. Rev. Entomol. **1**, 379-402 (1956). Palo Alto, Calif.
51. ULLYETT, G. C. Mortality factors in populations of *Plutella maculipennis* Curtis (Tineidae: Lepidoptera) and their relation to the problem of control. S. African Dept. Agr. Forest Entomol. Mem. **2**, 77-202 (1947).
52. UVAROV, B. P. Insects and climate. Trans. Entomol. Soc. London, **79**, 1-247 (1931).
53. WELLINGTON, W. G. Conditions governing the distribution of insects in the free atmosphere. I. Atmospheric pressure, temperature and humidity. Can. Entomologist, **77**, 7-15 (1945).
54. WELLINGTON, W. G. Conditions governing the distribution of insects in the free atmosphere. IV. Distributive processes of economic significance. Can. Entomologist, **77**, 69-74 (1945).
55. WELLINGTON, W. G. Air-mass climatology of Ontario north of Lake Huron and Lake Superior before outbreaks of the spruce budworm, *Choristoneura fumiferana* (Clem.) and the forest tent caterpillar, *Malacosoma disstria* Hbn. (Lepidoptera: Tortricidae: Lasiocampidae). Can. J. Zool. **30**, 114-127 (1952).
56. WELLINGTON, W. G. Atmospheric circulation processes and insect ecology. Can. Entomologist, **86**, 312-333 (1954).
57. WELLINGTON, W. G. Weather and climate in forest entomology. Meteorol. Monogr. **2**, 11-18 (1954).
58. WELLINGTON, W. G., FETTES, J. J., TURNER, K. B., and BELYEA, R. M. Physical and biological indicators of the development of outbreaks of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. J. Research, D, **28**, 308-331 (1950).

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## THE DIURNAL ACTIVITY OF THE LARGER INVERTEBRATES AT THE SURFACE OF LAC LA RONGE, SASKATCHEWAN<sup>1</sup>

J. H. MUNDIE<sup>2</sup>

### Abstract

Horizontal hauls were made with a pair of large plankton nets at the surface of a large lake to determine the 24-hour emergence cycle of chironomid midges rising from different depths and to investigate the occurrence of the larger invertebrates at the surface. Chironomid pupae ascend mainly in the hours of darkness and emergence may be immediate, as in *Psilotanytus rufovittatus*, or delayed for several hours, as in *Procladius choreus*. A migration to the surface at night is demonstrated for *Mysis relicta* and the amphipods *Pontoporeia affinis* and *Hyalella asteca*. Chironomid larvae and a variety of other invertebrates also occur at the surface. The findings show that many benthic animals are less static in their distribution than is commonly accepted.

### Introduction

The use of submerged traps for estimating populations of chironomid midges (Chironomidae = Tendipedidae) emerging from lakes has several disadvantages (10) and there is need for a more efficient method, especially one usable on large lakes of low productivity where emerging midges may be too sparse to be sampled adequately with traps. The present work investigates the possibilities of large coarse plankton nets for collecting ascending pupae. The nets were hauled horizontally just below the surface over specific depths of water for a selected distance at a uniform rate. It was hoped that hourly hauls throughout 24 hours could establish the periodicity of emergence of a species from any particular depth.

Initial work on a relatively small productive lake in the English Lake District led to the design of the net used on Lac la Ronge, and showed that the bulk of emergence took place between evening and morning. It also demonstrated that some invertebrates such as chironomid larvae and May fly nymphs, which are benthic in day time, were frequently present near the water surface at night. In a less productive lake these animals, being less common, would be more difficult to detect in the water. A second aim of this study, therefore, was to ascertain whether invertebrates which are usually considered as benthic could be found near the water surface at night, and, if so, to investigate the regularity of their occurrence.

<sup>1</sup>Manuscript received July 6, 1959.

Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask., and the Freshwater Biological Association, Ferry House, Far Sawrey, Ambleside, Westmorland, England. This work was supported by a fellowship from the National Research Council. Acknowledgment is made to the Council of the British Freshwater Biological Association for granting leave of absence.

<sup>2</sup>Department of Biology, University of Saskatchewan. Present address: Freshwater Biological Association, Ambleside, Westmorland, England.

### Lac la Ronge

A general account of Lac la Ronge, to which the reader is referred for details of morphometry and biology, is given by Rawson and Atton (12). The lake lies in central Saskatchewan and has an area of 500 sq. miles (Fig. 1). The southern region, where most of the sampling was done, lies south of the Pre-Cambrian Shield and is in the middle range of productivity. About 70% of the floor of this area lies below 0-15 m of water. Thermal stratification

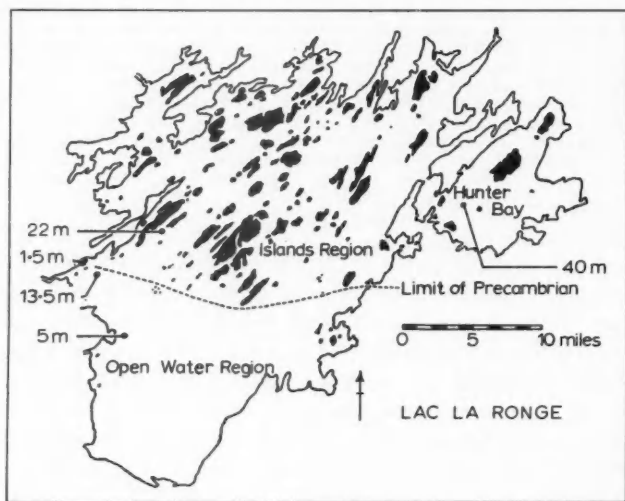


FIG. 1. Sketch map of Lac la Ronge showing main areas. Sampling stations are indicated by depth marks.

develops, often intermittently, in July and August, with the thermocline between 10 and 20 m. The highest surface temperature is about 20° C and the bottom dissolved oxygen values fall to 4 p.p.m. The remaining, more northerly, part of the lake lies on the Shield and includes Hunter Bay (area 50 sq. miles) which has the cold unproductive character of the more northern Saskatchewan lakes. The lake is free of ice from about mid-May to mid-November.

### Method

Initial experiments showed that a single net, floated so that it was half out of the water, caught, in addition to pupae, adult female midges as they laid eggs. A second net was therefore attached immediately below this one, which would catch only pupae. Plankton nets have been used previously for sampling chironomids (9) and double nets for zooplankton (3).

The nets were of nylon with 30 meshes per linear in. (aperture 0.75 mm) and were attached by canvas to a duralumin frame, which formed the mouth,

measuring 75 cm  $\times$  38 cm (Fig. 2). The total length of canvas and net was 115 cm. The buckets were 250-ml polyethylene jars with net bottoms and were screwed into threaded duralumin collars. The nets were mounted on a "Dexion" steel frame on each side of which was a cylindrical float.

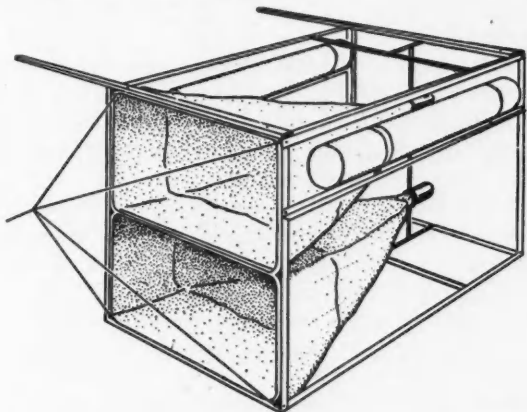


FIG. 2. Sampling apparatus consisting of two coarse-meshed plankton nets mounted one above the other in a rectangular steel frame. The cylinders at the sides are floats. When the apparatus is in use the upper half of the top net is out of the water. The arms extending to one side keep the nets a constant distance from the side of the boat.

All hauls were made at the surface over different depths of water from a 28-ft motor launch. The apparatus was tied amidships on the port side to a horizontal arm close to the water, and two spars on the top of the frame kept it a constant distance from the boat. The nets were hauled alongside the boat so that they were ahead of its wake and readily procurable to be lifted aboard. Hauls were made between two dry-battery lights mounted on moored buoys stationed about 600 m apart. Each haul lasted 10 minutes, the speed being about 1 m per sec. Sampling was generally begun at 7.00 p.m. Standard Time, and a haul was made every hour until 6.00 a.m.

Sampling was done with the wind, as, against it, it was difficult to hold a straight course at slow speed. If wave action was more than light, the catch could be washed forward out of the net. Calm conditions, therefore, were a prerequisite and were also required to ensure that the drift of fauna into the sampling area from adjacent depth-zones was minimal. Very calm conditions did not always hold throughout the 11-hour period, and, because of the size and exposure of the lake, only 8 nights were suitable for all-night sampling in the period mid-July to the end of August in 1958. The need for calm weather is a limitation of the method.

The net was assumed to filter efficiently on account of its coarse mesh.

The mean depths of the sampling stations and the dates of sampling are given in the histograms. The station at 1.5 m, a sheltered bay, was sampled once, the others were each sampled on 2 nights. Hunter Bay was visited on

August 19 when several hauls were made over 15 m, 22 m, and 40 m, and on June 6 of the following year (1959) when hauls were made with a single net above 13 m, 30 m, and 40 m.

## Results

### *Emerging Chironomidae*

The chironomids caught in greatest numbers were Tanypodinae.

*Psilotanypus rufovittatus* v.d.W., a species which has one generation a year in England (11), is abundant at 5 m and occurs down to at least 22 m. The pupae were caught from 8.00 p.m. until 6.00 a.m., the greatest numbers at 10.00 p.m. and 11.00 p.m. (Fig. 3).

The catches in the top net were approximately half those of the lower one. Since the top net is half out of the water, and therefore filters half the volume of water of the lower one, this result is to be expected, assuming a uniform density of pupae in the upper 57 cm of water.

Although this species is one of the commonest chironomids in the lake this would not be suspected from observations of swarming adults on the lake shore, or from catches of adults at lights.

No specimens of *Psilotanypus* were taken at Hunter Bay.

*Proclodius choreus* Meig., which is closely related to, or the same as, *P. culiciformis* L., is one of the most abundant insects in the lake. Some adults examined provided a series showing characters grading to those of *P. crassinervis* Zett., a situation which has been found elsewhere (11). The pupae of the two forms, and of another species occasionally found as an adult, were indistinguishable.

The highest numbers of *P. choreus* caught during all-night sampling were obtained on August 14 above 13.5 m. Here the lower net collected fairly uniform numbers (Fig. 4) between 9.00 p.m. and 3.00 a.m., demonstrating a uniform density of ascent throughout the night. The top net, however, collected increasing numbers until 3.00 a.m. Similar catches were obtained on August 7. The histograms for 5 m show highest catches at 1.00 a.m. and 2.00 a.m. on August 9, the top net again taking the highest numbers. This might be explained by postulating a drift of pupae on the water surface from a shallower, more productive zone into the sampling area. Since, however, no corresponding increase in numbers in the top net is shown for *Psilotanypus* this possibility is dismissed. A more likely explanation seems to be a delay in emergence of the adults lasting several hours so that the pupae accumulate at the surface. Evidence in support of this is that pupae brought ashore the morning after capture at 10.00 p.m. over 13.5 m did not produce adults for 18–24 hours after capture. On the lake they would be carried to shallow water in this time.

On August 31 two hauls were made at the 13.5-m station at 10.10 p.m. and 10.30 p.m. The first gave 249 individuals of *P. choreus* in the top net and 40 in the lower. The second gave 307 and 41. These high numbers suggest that the seasonal mode of emergence was at the end of August, and if this was

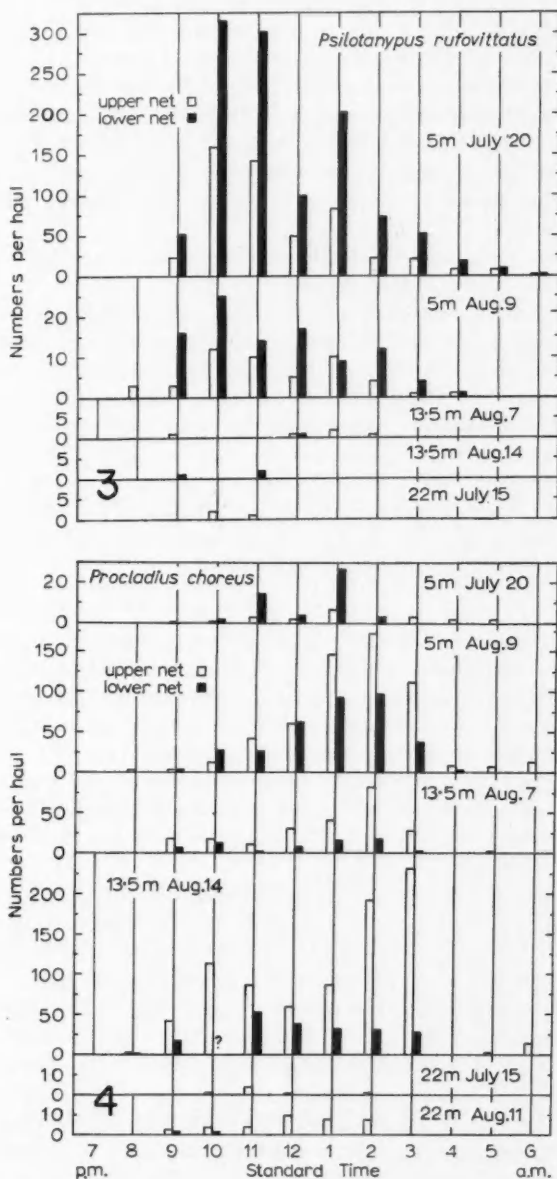


FIG. 3. Catches of *Psilotanytus rufovittatus* at the surface above various depths.  
 FIG. 4. Catches of *Procladius choreus* at the surface above various depths.

so, the data in Fig. 4 were obtained 2 or more weeks before this. If the species has its seasonal mode at this time it probably has only one generation a year at 13.5 m. In England it has two generations at 6.8 m (11).

Only a few specimens were caught at Hunter Bay on August 19.

The commonest *Chironomus* species in the lake are *C. anthracinus* Zett., *C. plumosus* L., and *C. decorus* Joh. Over 13.5 m on August 14 a total of 44 individuals of *C. decorus* was caught between 10.00 p.m. and 2.00 a.m. Over 22 m, a few specimens of *C. plumosus* were taken between midnight and 2.00 a.m.

A dozen other species of chironomid were found as pupae, but in insufficient numbers to show modes of emergence.

The chironomid cast pupal skins accumulate on the surface in the course of the night. From the numbers caught the absolute number of pupae emerging per sq. m per hour could be derived if no addition or subtraction of skins takes place owing to drift. This condition, however, does not hold, even on very calm nights. For example, on August 14, over 13.5 m, the numbers of *Chironomus* pupal skins in the top net at midnight, 1.00 a.m., and 2.00 a.m. were 7200, 3000, and 9600. On July 20, over 5.0 m, the number of skins of *Glyptotendipes* pupae dropped from 650 at midnight to 460 at 1.00 a.m. although *Glyptotendipes* continued to emerge until 4.00 a.m. Further studies are therefore required to establish absolute numbers.

#### Other Insects

Chironomid larvae were taken over all the depths sampled, except at Hunter Bay. Most occurred over 5 m (Fig. 5) where early and late instars were found consisting of 60% Chironomini, 25% Tanypodinae, and 15% Orthocladinae. On August 14 a total of 16 larvae, all Chironomini, was collected between 9.00 p.m. and 3.00 a.m. over 13.5 m, and on August 11, over 22 m, six fourth instar *Chironomus* larvae were taken between 10.00 p.m. and 1.00 a.m. These findings show that an unknown fraction of the benthic population moves into the water at night, but the cause of the movement, and whether it is random or directional, is unknown.

Ceratopogonid pupae of the *Palpomyia* group were found above 5 m. On July 20, 37 specimens were collected, all in the top net, between 10.00 p.m. and 6.00 a.m. The largest catch, of 13 pupae, was taken at 11.00 p.m. along with four larvae, one of which was in the process of pupating. Larvae also occurred in small numbers, although able to pass through the net, over 13.5 m and 22 m at times ranging from 9.00 p.m. to 7.00 a.m. The larvae can evidently pupate at the surface although many may do so on reaching the shore (14).

Adult ceratopogonids attracted to the gasoline lights on the boat during the hours of darkness were *Palpomyia aldrichi* (Malloch), *P. armatipes* Wirth, *Johannsenomyia* sp., and *Dasyhelea* sp.

Over 5 m on July 20 the distribution of nymphs of the corixid *Sigara trilineata* (Prov.) was as shown in Fig. 5. Over 1.5 m, on August 28, nymphs amounting to 177 in one haul were obtained, but these were associated with

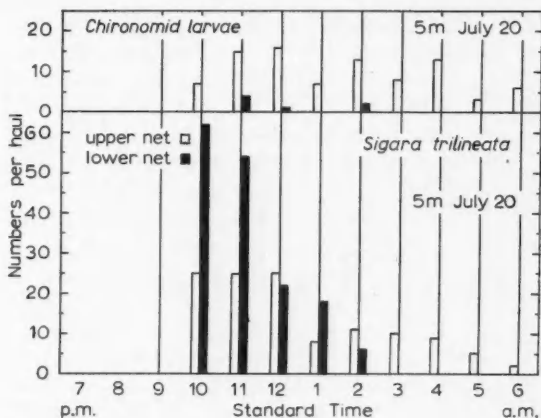


FIG. 5. Catches of chironomid larvae and of nymphs of *Sigara trilineata* at the surface above various depths.

vegetation (mainly *Myriophyllum*) and the catches were not related to the surface activity of the insects. The adult corixids found at this station were *Trichocorixa borealis* Sailer, *Palmarcorixa buenoi* Abbott, *Dasycorixa hybrida* (Hungerford), *Cenocorixa dakotensis* (Hungerford), *Hesperocorixa vulgaris* (Hungerford), *Sigara decoratella* (Hungerford), and *Sigara trilineata* (Provancher).

Nymphs of *Caenis latipennis* Banks and *Hexagenia oculata* Walk. (Ephemeroptera) were found in small numbers over all depths sampled, and larvae, in cases, of *Triaenodes frontalis* Banks (Trichoptera) occurred over 5 m and 13.5 m.

#### Larger Crustacea

##### *Mysis relicta* Lovén

The records of diurnal movements of this species have been reviewed by Holmquist (5). Nearly all specimens found in Lac la Ronge in July and August measured 8–9 mm in length, very few mature ones (20 mm) being caught. Over the depths sampled *Mysis* was not taken before 9.00 p.m. or after 2.00 a.m. and the main surface activity was at midnight (Fig. 6). Although the lower net filtered twice the volume of water of the upper one, the ratio of the number caught in it to that in the upper net for all collections on the main lake was 1:1.7. The animals were therefore strongly concentrated in the top 19 cm of water.

Specimens were taken in small numbers above 1.5 m at a water temperature of about 20° C.

There appear to be seasonal differences in the extent of the migration, as shown by the catches of July 15 and August 11, and the mode at the surface may fluctuate. On August 11, for example, over 22 m, the greatest catch was at 10.00 p.m. At Hunter Bay on August 19, greatest numbers were found at

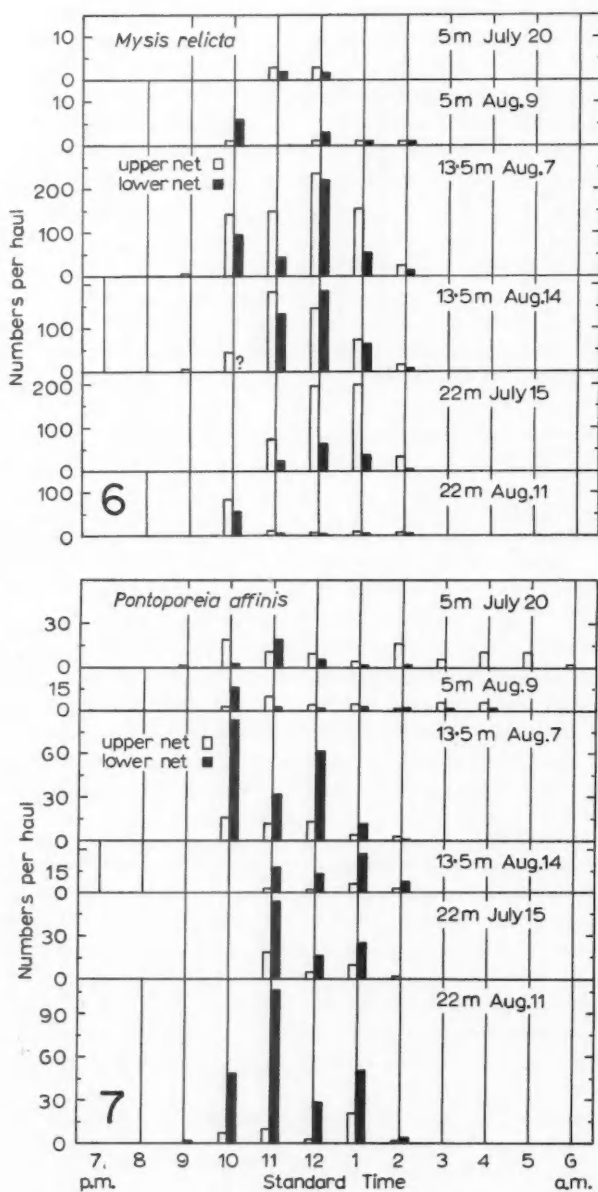


FIG. 6. Catches of *Mysis relicta* at the surface above various depths.  
 FIG. 7. Catches of *Pontoporeia affinis* at the surface above various depths.

10.30 p.m. over 40 m when the top net caught 65 specimens, and the bottom net 116. At 12.30 a.m., the top net took 23, and the bottom net 21 specimens. At 1.30 a.m. the numbers were 9 and 12.

The migration of *Mysis* is not confined to the older instars. On June 6 of the following year (1959), three hauls were made with one net, just below the surface, on Hunter Bay. At 11.30 p.m. over 40 m, three *Mysis* were taken; at midnight over 30 m, 60 were caught; and at 12.15 a.m. over 13 m, 434 were caught. All of these measured 4.5 mm in length.

It is likely, in view of the distinctness of these findings, that the vertical migrations of *Mysis* in lakes are more common than is generally recognized, and that Southern and Gardiner's observation (13) on *Mysis* in Lough Derg—"in both deep and shallow water it leaves the bottom at night and may be captured at any level right up to the surface"—is of wide application.

*Pontoporeia affinis* Lindström

Larkin (8) observed a diurnal migration of *P. affinis* in 1–2 m of water at the edge of Great Slave Lake. This, however, may be much more extensive. The Lac la Ronge catches (Fig. 7) show that fairly high numbers occurred near the surface over depths ranging from 5 to 22 m. The deep-water samples gave highest numbers between 10.00 p.m. and 1.00 a.m., no specimens being taken after 2.00 a.m. The lower net caught more than twice as many individuals as the upper except over 5 m.

In the southern region of the lake *P. affinis* is present on the bottom in numbers of about 40 per sq. m at 22 m. In Hunter Bay it is the commonest macroscopic invertebrate and the numbers amount, at 40 m, to 1500 per sq. m. Nevertheless, three hauls made there between 10.30 p.m. and 1.30 a.m. over 40 m on August 19, 1958, gave a total of only six specimens, probably because of the greater depth. On June 6 of the following year three hauls were made with one net, just below the surface, on Hunter Bay. At 11.30 p.m. over 40 m no *Pontoporeia* were taken; at midnight over 30 m 3 were caught; and at 12:15 p.m. over 13 m, 17 were caught.

*P. affinis* has been recorded at the surface of Ungava Bay (4). This record may refer, however, to a form distinct from Lindström's species (1).

*Hyalella azteca* Saussure

*Hyalella azteca* Saussure is common below shallow water in the main lake, particularly in sheltered bays, and occurs occasionally at 22 m. The hauls above 5 m and 13.5 m gave small catches which suggest a diurnal migration with no clear mode, and with the highest numbers usually in the top net (Fig. 8).

In shallow bays rooted vegetation may support more amphipods than does the mud (2). Consequently the hauls over 1.5 m on August 28, in a bay containing *Myriophyllum*, pieces of which were picked up by the net, gave catches of up to 730 individuals of *H. azteca*. These had no bearing on surface activity.

*Gammarus pulex* (L.) is recorded as pelagic in a lake at high altitude in Indian Tibet (6).

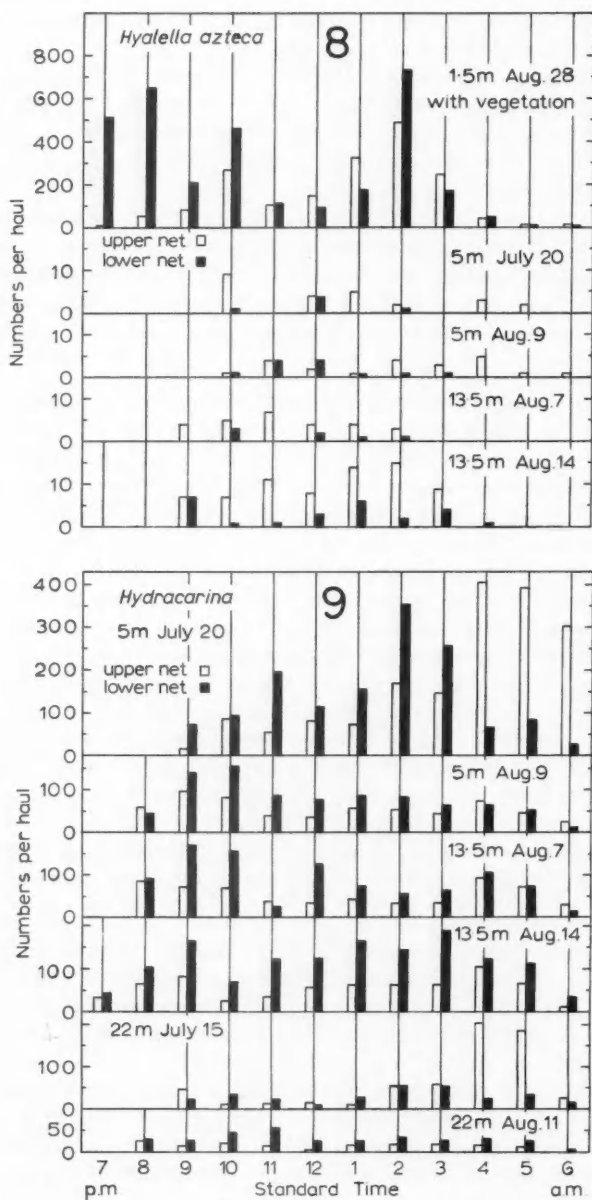


FIG. 8. Catches of *Hyalella azteca* at the surface above various depths.  
 FIG. 9. Catches of *Hydracarina* at the surface above various depths.

### Other Animals

Hydracarina: Water mites occurred in all the samples, the greatest numbers being taken over 5 m (Fig. 9). Catches showed a uniform density of mites in the top 57 cm at most times but there is evidence of a move upwards between 4.00 a.m. and 6.00 a.m. Several species occurred but *Piona* sp. was conspicuously the commonest.

The leech *Pisicola punctata* (Verrill) was found most commonly above 1.5 m where 50 were taken on August 28 between 7.00 p.m. and 4.00 a.m., 36 being in the lower net. Above 5 m, only 6 were taken in 2 nights; and over 13.5 m 2 nights' sampling gave 31 specimens of which 30 were in the top net. Only three were found over 22 m on 2 nights. The species, therefore, apparently searches for its hosts in the open lake close to the surface at night.

The fish most frequently caught were nine-spined sticklebacks (*Pungitius pungitius* L.) which were found in small numbers throughout the night over all depths sampled. On August 31, two hauls at 10.10 p.m. and 10.30 p.m. over 13.5 m gave a total of 20 sticklebacks, 19 of which were in the lower net. Spottail minnows (*Notropis hudsonius* Clinton) were common in hauls over 1.5 m. The use of townets for sampling young fish near the surface is discussed by Johnson (7).

### Concluding Remarks

The above findings, although based on only a few nights' sampling, are sufficient to show that the method can be used, with the limitation of suitable weather conditions, to determine the 24-hour emergence cycle of chironomids rising from different depths in a large lake in which trapping might be impracticable. This implies that sampling on selected dates throughout the whole period of emergence could establish the seasonal distribution and number of generations of the species found. In the course of the work it became evident that some chironomids, such as species of *Tanytarsus*, which were abundant in swarms on the shore, were not being caught in corresponding numbers in the hauls, in spite of the range of depths sampled. It is likely, therefore, that the substrata below the sampled areas did not support many of these species and that a complete account of emerging pupae would require sampling over different types of substrata at the same depths.

The catches of the larger invertebrates, other than pupae, show that these, although sparse, can also be sampled adequately by making hauls of appropriate duration. The method should therefore be of value on any lake for determining which invertebrates most commonly come to the surface, or, with the choice of nets of suitable mesh, for analyzing the detailed movements of particular species.

Any consideration of the invertebrates near the surface demands the distinction between those which actively move up from the bottom, or the lower waters, either diurnally, or as a result of random or at least less markedly directional movement, and those which are passively carried there by water movements. This study does not touch upon the causes of the surface activity

of the larger invertebrates, but merely draws attention to its extent. This implies that some animals which are members of the benthos are less static in their distribution, and more independent of benthic conditions, than is commonly accepted.

### Acknowledgments

Mr. S. Kyba took part in most of the sampling; his help and that of Mr. J. MacNeill was indispensable. My best thanks go also to Mr. R. Morris for assistance in sorting material, to Mr. A. R. Brooks for identifying corixids, to Dr. L. G. Saunders for identifying ceratopogonids, and to Dr. D. S. Rawson for facilities and help throughout the investigation.

### References

1. BOUSFIELD, E. L. Freshwater amphipod crustaceans of glaciated North America. *Can. Field-Naturalist*, **72**, 55-113 (1958).
2. CLEMENS, H. P. Life cycle and ecology of *Gammarus fasciatus* Say. Ohio State University; Franz Theodore Stone Instit. Hydrobiol. **12**, 1-63 (1950).
3. COLMAN, J. S. and SEGROVE, F. The tidal plankton over Stoupe Beck Sands, Robin Hood's Bay (Yorkshire, North Riding). *J. Anim. Ecol.* **24**, 445-462 (1955).
4. DUNBAR, M. J. The amphipod Crustacea of Ungava Bay, Canadian eastern Arctic. *J. Fisheries Research Board Canada*, **11**, 700-798 (1954).
5. HOLMQUIST, C. Problems of marine-glacial relicts on account of investigations of the genus *Mysis*. Lund. 1959.
6. HUTCHINSON, G. E. Limnological studies in Indian Tibet. *Intern. Rev. ges. Hydrobiol.* **35**, 134-177 (1937).
7. JOHNSON, W. E. On the distribution of young sockeye salmon (*Oncorhynchus nerka*) in Babine and Nilkitkwa Lakes, B.C. *J. Fisheries Research Board Canada*, **13**, 695-708 (1956).
8. LARKIN, P. A. *Pontoporeia* and *Mysis* in Athabaska, Great Bear, and Great Slave Lakes. *Bull. Fisheries Research Board Canada*, **78**, 1-33 (1948).
9. LEWIS, D. J. Observations on Chironomidae at Khartoum. *Bull. Entomol. Research*, **48**, 155-184 (1957).
10. MUNDIE, J. H. Emergence traps for aquatic insects. *Mitt. intern. Ver. Limnol.* **7**, 1-13 (1956).
11. MUNDIE, J. H. The ecology of Chironomidae in storage reservoirs. *Trans. Roy. Ent. Soc. (London)*, **109**, 149-232 (1957).
12. RAWSON, D. S. and ATTON, F. M. Biological investigation and fisheries management at Lac la Ronge, Sask. Fisheries Branch, Dept. Nat. Resources, Sask. 1953.
13. SOUTHERN, R. and GARDINER, A. C. The seasonal distribution of the crustacea of the plankton in Lough Derg and the River Shannon. *Fisheries, Ireland, Sci. Invest.* **1**, 1-170 (1926).
14. WEEREKOON, A. C. J. On the behaviour of certain Ceratopogonidae (Diptera). *Proc. Roy. Ent. Soc. (London) A*, **28**, 85-92 (1953).

## SEPARATION OF THE PROTEIN CONSTITUENTS OF THE LARVAL DIETS OF THE HONEYBEE BY CONTINUOUS PAPER ELECTROPHORESIS<sup>1</sup>

J. E. J. HABOWSKY<sup>2</sup> AND R. W. SHUEL<sup>3</sup>

### Abstract

The protein constituents of the larval diets of queen and worker honeybees were separated by continuous paper electrophoresis. The electrophoretic patterns of royal jelly of any age and the early worker diet were similar and comprised five ninhydrin-reactive bands or fractions. Fraction 1 (nearest the cathode) contained lysine as a free amino acid. Fractions 3 and 4 appeared to be complex polypeptides. Alanine, asparagine, aspartic acid, glutamic acid, glycine, histidine, isoleucine and/or leucine, lysine, phenylalanine, threonine, tyrosine, valine, and an unidentified substance were found in chromatograms of the acid hydrolyzate of fraction 3; the hydrolyzate of fraction 4 contained the same amino acids except for threonine. Fractions 2 and 5 were not characterized. Electrophoresis of the diet of worker larvae older than 3 days showed a pronounced fading of all bands, attributable to the dilution of the solids by the addition of honey which occurs at this time. There appeared to be no qualitative differences between the protein fractions of royal jelly and worker diet which would account for the differentiation of female honeybees into queens and workers. The decrease with age in the percentage of protein in the worker diet may be significant.

### Introduction

Female dimorphism in the honeybee, *Apis mellifera* L., is a function of nutrition (7). During the first 3 days of larval life all female larvae apparently are capable of becoming either queens or workers. At this stage all larvae are fed on pure secretions of the hypopharyngeal glands of nurse bees (7,10). The secretion fed to queen larvae differs from that fed to worker larvae (8). After the third day the diet of the worker larva is modified by additions of honey and pollen to the secreted material, whereas queen larvae continue to be nourished on a pure secretion (7). Larvae destined to become queens are characterized by a faster growth rate after the second or third day (5).

In this study the protein fractions of foods given to larvae of various ages were examined by continuous paper electrophoresis for differences which might relate to dimorphism. Some of the constituent amino acids were tentatively identified by paper chromatography.

The secretion fed to queen larvae is universally known as "royal jelly". The following terms will be used to describe the food supplied to worker larvae: "worker jelly" will denote the pure secretion fed to young worker larvae, "modified jelly" the food supplied to larvae older than 3 days, which contains an admixture of honey and pollen. The general term "worker diet" will include both worker jelly and modified jelly (8).

<sup>1</sup>Manuscript received July 22, 1959.

Contribution from the Apiculture Department, Ontario Agricultural College, Guelph, Ontario. This paper represents part of a thesis submitted for the degree of M.S.A. by the senior author.

<sup>2</sup>Formerly graduate student, Apiculture Department, Ontario Agricultural College; presently graduate student, Department of Zoology, University of Toronto.

<sup>3</sup>Apiculture Department, Ontario Agricultural College.

## Materials and Methods

### *Collection of Samples*

Samples of royal jelly and worker diet were collected from cells containing larvae, at 6-hour intervals of larval growth. Since the amount obtainable from a single cell is rather minute, a collection usually represented several colonies and different combs of brood within a colony. In order to collect royal jelly, 30-hour-old larvae were placed in artificial queen cells, and the jelly fed to them by nurse bees was removed from the cells by aspiration at the end of a specified period (9). Worker diet was collected in a similar manner from brood combs containing larvae of known ages. All samples were stored at  $-17^{\circ}\text{C}$  immediately after collection. Sample age, as used hereafter, will denote the age of the oldest secretion in the sample.

### *Electrophoretic Apparatus*

The Shandon continuous paper electrophoresis apparatus (catalogue No. 2522) was used with several modifications. The buffer supply to the side electrodes was increased to 30 liters to permit a 24-hour continuous operation without refilling. To prevent excessive heating of the paper and the consequent evaporation of the buffer and sample, a plastic cooling plate through which cold water could be circulated was installed behind the paper electrophoresis curtain. The cellophane bags containing the platinum wire side electrodes, and through which the buffer solution flows continuously, were replaced by artificial casings of the type used to wrap sausages. These lasted for 3 to 4 weeks of continuous use. The bottom electrodes were not used.

In the unmodified apparatus a rate of buffer flow sufficient to keep the electrode casings distended had been found to drain the buffer reservoir too quickly. The rate of flow was therefore reduced by inserting a section of glass tubing tapering to capillary dimensions at one end into the outlet tube of the electrode bags.

The system for supporting the paper and the side electrodes was also modified since the turgor developed in the bags tended to bend the paper backward. Plastic strips were attached to the cooling plate immediately behind the electrode bags, the electrophoresis paper being trimmed to accommodate the clamps.

Finally, the wick used to apply the sample to the paper was replaced by a motor-operated syringe (Fig. 1). The assembly was mounted independently of the electrophoresis cabinet. A 1/150-hp motor turned a small wheel with an eccentrically attached rod which acted as a ratchet, engaging a toothed wheel which turned a screw through a nut. The screw in turn activated the piston of the syringe, which was inserted through a hole drilled in the top panel of the cabinet. The needle of the syringe rested against the top of the electrophoresis paper. A delivery rate of 1 ml of sample in 40 hours was achieved by using a 40-minute period for one revolution of the toothed wheel, a screw with 9.5 threads per cm, and a 1-ml standard tuberculin syringe with a 25-gauge needle.

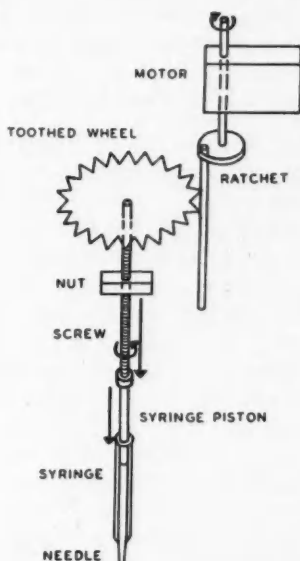


FIG. 1. System for applying sample to paper for electrophoretic separation.

Standard operating conditions were adopted for all electrophoretic separations. The current was adjusted to 11 milliamperes at an E.M.F. of 80 volts. A fairly heavy paper with a medium flow rate, Whatman No. 3 MM, was used. The water circulating through the cooling plate was maintained at 15°C. The buffer mixture, which consisted of 20.6 g of sodium barbital and 150 ml of 0.1 *N* HCl diluted to 1 liter (4), had a pH of 8.5. The buffer solution for the paper curtain was freshly prepared for each operation. The pH of the buffer solution supplying the side electrodes was checked at intervals and found to remain in the range 8.3–8.5. The apparatus was equilibrated for 2 hours before the sample was applied to the paper. The syringe assembly could be fitted into place without opening the cabinet.

#### *Preparation of Samples for Separation*

After the sample was warmed to 20°C and mixed well, the percentage of total solids was determined with an Abbé refractometer. Two hundred milligrams (containing from 65 to 75 mg of solid materials, depending on the sample) were then mixed with 2 ml of water and sufficient 1 *N* NaOH to raise the pH to 9.0. Essentially all of the sample was then in solution. Good separations were achieved at pH's up to 11.0. Filtration of the sample was found unnecessary.

#### *Development of Electrophoretograms*

The separated fractions migrated down the entire length of the paper in 20 hours. In this period 0.5 ml of the diluted sample were discharged from the syringe. The paper was oven-dried at 90–95°C for 30 to 40 minutes,

sprayed with 0.3% ninhydrin in 95% ethanol (3), and replaced in the oven for 3 minutes. The lightly colored bands were then saturated with spray and the paper stored in darkness at 20° C to develop the colors fully. When fully developed the colors were stable for several months.

#### *Collection and Treatment of Fractions for Chromatography*

Fractions separated on the electrophoresis paper were collected in the receiving vials for chromatography. To avoid excessive dilution of the biological material by the buffer solution, the following procedure was adopted: A 1 ml volume of sample was applied to the paper. Shortly before the separated fractions had reached the serrated edge of the paper which dipped into the collecting vials (after about 18 hours) the vials were emptied of the buffer solution which had accumulated. They were then replaced in their original positions and the electrophoretic separation was continued for an additional 22 hours. The vials containing the various fraction were identified by spraying the paper in the usual manner. Each fraction was recovered from the vials into which it had been delivered, evaporated *in vacuo* and hydrolyzed under reflux with HCl for 20 hours (3). After removal of excess HCl, the residues were dried, taken up in water, and filtered. The filtrates were evaporated to dryness and weighed. Recovery varied from 10 to 20 mg of solids per fraction. Most of the sodium barbital was destroyed by hydrolysis.

For chromatography of free amino acids, fractions were evaporated *in vacuo* and the residues weighed. Yields varied from 100 to 200 mg, much of which was sodium barbital. The residue was dissolved in water.

TABLE I

The amino acid composition of the acid hydrolyzates of fractions 3 and 4 of 48-hour worker jelly

| Amino acid                | Fraction 3 |         | Fraction 4 |         |
|---------------------------|------------|---------|------------|---------|
|                           | 1st run    | 2nd run | 1st run    | 2nd run |
| Alanine                   | +          | +       | +          | +       |
| Asparagine                | +          | +       | +          | +       |
| Arginine                  | -          | -       | -          | -       |
| Aspartic acid             | +          | +       | +          | +       |
| Cystine                   | -          | -       | -          | -       |
| Glutamine                 | -          | -       | -          | -       |
| Glutamic acid             | +          | +       | +          | +       |
| Glycine                   | +          | +       | +          | +       |
| Histidine                 | +          | +       | +          | -       |
| Hydroxyproline            | -          | -       | -          | -       |
| Isoleucine and/or leucine | +          | +       | +          | +       |
| Lysine                    | +          | +       | -          | +       |
| Methionine                | -          | -       | -          | -       |
| Ornithine                 | -          | -       | -          | -       |
| Phenylalanine             | +          | +       | -          | +       |
| Proline                   | -          | -       | -          | -       |
| Serine                    | -          | -       | -          | -       |
| Taurine                   | -          | -       | -          | -       |
| Threonine                 | +          | -       | -          | -       |
| Tyrosine                  | +          | +       | +          | +       |
| Valine                    | +          | +       | +          | +       |
| Unidentified              | +          | +       | +          | +       |

### Chromatography

Descending two-dimensional chromatograms were developed in a Chromatocab (Research Equipment Corporation) using solvent mixtures of 1-butanol: water: acetic acid in the ratio 25:25:6 (v/v/v) and phenol:water in the ratio 4:1 (v/v) with an addition of 0.04% 8-quinolinol and in the presence of 0.3% ammonia (3). Whatman No. 1 paper was used.

Reference chromatograms of 22 amino acids (Table I) were first prepared, using 5  $\mu$ g of each amino acid. After development in the butanol - acetic acid solvent the paper was dried at 60-70° C for 1 hour, replaced in the Chromatocab at right angles to its original position, and developed in the phenol solvent. After air-drying, the paper was sprayed with the ninhydrin reagent and stored in darkness at 20° C for full color development.

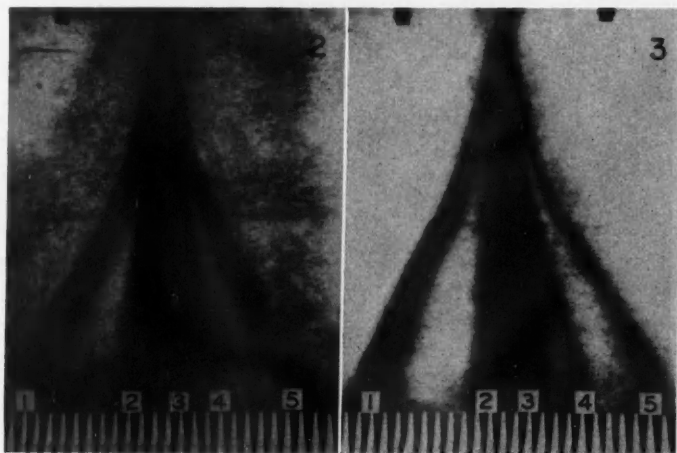
The fractions obtained by electrophoresis of the larval diets were chromatographed in the same way. For hydrolyzed fractions, 10-15  $\mu$ g aliquots were used, and for unhydrolyzed fractions, in which the proteinaceous material was diluted by residual buffer salts, 20-30  $\mu$ g aliquots were used.

### Results and Discussion

#### Paper Electrophoresis

Sixteen electrophoretic separations of royal jelly and 29 of worker diet, representative of the diets fed to queen and worker larvae between 30 and 132 hours of age, were made.

A typical electrophoretic separation of royal jelly is shown in Fig. 2. The fractions are numbered from the left (cathode) side of the paper. Five distinct bands appeared in all samples from 12 to 100 hours old except in one or two instances when the fourth band was absent. This missing band appeared when the sample size was increased to 400 mg wet weight, or about 130 mg dry



FIGS. 2 and 3. Electrophoretic separation of protein constituents of 42-hour royal jelly (Fig. 2) and of 42-hour worker jelly (Fig. 3). Fraction 1 is nearest the cathode.

weight. A representative electrophoretogram of worker diet prior to the third day (worker jelly) is shown in Fig. 3 and is seen to be identical with the electrophoretogram of royal jelly in Fig. 2. Figure 4 illustrates the characteristic electrophoretic pattern of older samples of worker diet (modified jelly). The fourth, and sometimes the fifth band were missing, and the remaining bands were quite faint. Doubling the sample size restored the missing band. This change in the intensity of the electrophoretic bands of the protein fraction of worker diet is in accord with recent chemical analyses (8) which revealed a marked reduction in total protein beginning at about the third day of larval life. Ammon and Zoch (2) recently found four ninhydrin-reactive bands in electrophoretograms of a water extract of royal jelly.

*Electrophoresis of Dialyzed Royal Jelly*

A 70-mg sample of lyophilized 70-hour royal jelly which had been dialyzed for 6 days in an artificial sausage casing was separated electrophoretically. Only bands 3 and 4 appeared.

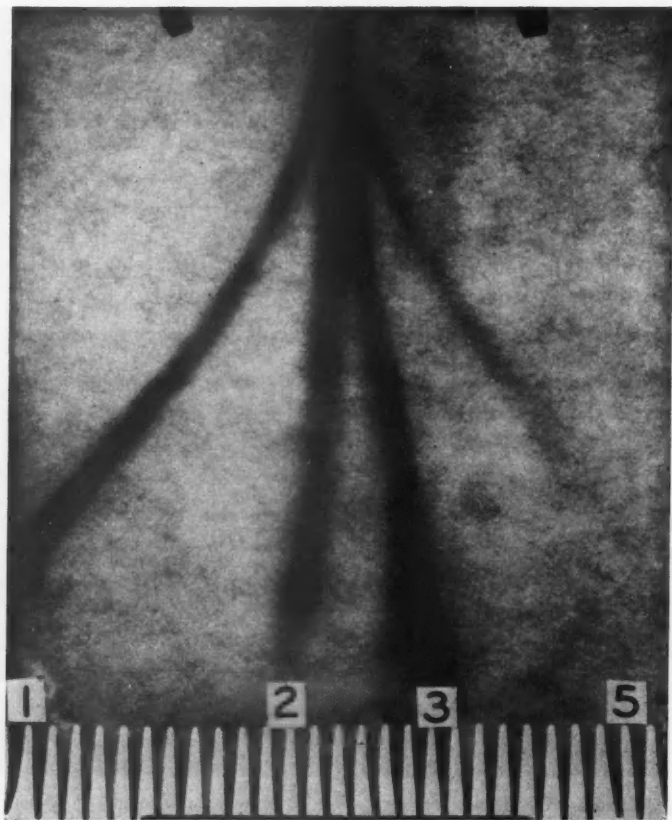


FIG. 4. Electrophoretic separation of 78-hour worker diet.

### *Chromatography*

The amino acids of the acid hydrolyzates of fractions 3 and 4 of a sample of 48-hour worker jelly, tentatively identified by their  $R_f$  values, are listed in Table I. Two runs were made on each fraction. Alanine, asparagine, aspartic acid, glutamic acid, glycine, histidine, isoleucine and/or leucine, lysine, phenylalanine, tyrosine, and valine were found in at least one instance in each of the two fractions. Threonine was found in fraction 3, but not in fraction 4. An unidentified ninhydrin-reactive substance which migrated close to aspartic acid appeared in the hydrolyzate of both fractions. As the fractions were not dialyzed prior to hydrolysis, some of the amino acids may have been present in the free state. Fraction 3 of 48-hour royal jelly contained the same amino acids as fraction 3 of worker jelly. Fraction 4 of royal jelly contained eight of the amino acids found in the corresponding fraction of worker jelly; glycine, isoleucine and/or leucine, and tyrosine did not appear. Since the electrophoretic migration of fraction 4 was the same for royal jelly as for worker jelly (Figs. 2 and 3), the missing amino acids may have been present at a concentration too low for detection. For some unknown reason, the second chromatogram of fraction 4 of royal jelly failed to give a color reaction with ninhydrin.

From the results of the electrophoresis of dialyzed material and of the chromatography, fractions 3 and 4 appear to have been complex polypeptides which were qualitatively similar.

No amino acids could be identified in the acid hydrolyzates of fractions 1, 2, and 5. Lysine was identified by co-chromatography as a free amino acid in all unhydrolyzed samples of fraction 1. Tests for free amino acids in the other fractions were negative. The high concentration of residual sodium barbital interfered with the ninhydrin tests (3). Difficulty was likewise encountered with alkaline hydrolysis; the test for tryptophane was negative, due either to its absence or to interference by ninhydrin.

A comparison of the amino acids listed above with those found in royal jelly by other investigators is not critical, as the constituents of fractions 2 and 5 were not identified. With the exception of asparagine, all the amino acids found in fractions 3 and 4 of royal jelly were found earlier as products of acid hydrolysis (1, 6, 11, 12). Many of the same amino acids were also found in the free state (2, 6). Combined amino acids not found in this study but reported by other workers include arginine and methionine (6, 12) and cystine, proline, and serine (6).

### **Conclusions**

The electrophoretic patterns of the protein fractions of royal jelly and worker jelly, both pure secretions of the hypopharyngeal glands of nurse bees, were very similar. In contrast to royal jelly, which evidenced no change with age, worker diet showed a pronounced weakening of the electrophoretic bands after the third day. This was probably due to the dilution of the protein by the addition of honey to the worker jelly (8). The chromatographic data, although incomplete, revealed no distinct qualitative differences between the

amino acid complements of 48-hour samples of the two secretions. Consequently, it appears unlikely that female dimorphism is attributable to qualitative variation in the protein fractions of the diet. Quantitative variation in protein might contribute both to differences in form and differences in growth rate (5).

### Acknowledgments

We wish to thank Professor G. F. Townsend, Dr. M. V. Smith, and other members of the Department of Apiculture, Dr. D. Waghorne, Department of Chemistry, and Dr. S. E. Dixon, Department of Entomology and Zoology, all of the Ontario Agricultural College, and Dr. S. Vesselinovitch, Ontario Veterinary College, for help in this study. We are indebted to the Ontario Research Foundation for financial assistance.

### References

1. AEPFLER, C. W. Tremendous growth force. *Gleanings in Bee Culture*, **50**, 151-153 (1922).
2. AMMON and ZOCH. Zur Biochemie des Futtersaftes der Bienenkoenigin. *Arzneimittel-Forsch.* **7**, 699-702 (1957).
3. BLOCK, R. J., DURRUM, E. L., and ZWEIG, G. A manual of paper chromatography and paper electrophoresis. Academic Press, Inc., New York. 1958.
4. GOMORI, G. Microscopic histochemistry. The University of Chicago Press, Chicago. 1952.
5. MELAMPY, R. M. and WILLIS, E. R. Respiratory metabolism during larval and pupal development of the female honeybee (*Apis mellifica* L.). *Physiol. Zool.* **12**, 302-311 (1939).
6. PRATT, J. J. and HOUSE, H. L. A qualitative analysis of the amino acids in royal jelly. *Science*, **110**, 9-10 (1949).
7. RIBBANDS, C. R. The behaviour and social life of honeybees. Bee Research Association, London. 1953.
8. SHUEL, R. W. and DIXON, S. E. Studies on the mode of action of royal jelly in honeybee development. II. Respiration of newly emerged larvae on various substrates. *Can. J. Zool.* **37**, 803-813 (1959).
9. SMITH, M. V. Some facts about royal jelly production. *Gleanings in Bee Culture*, **82**, 713-715 (1954).
10. SNODGRASS, R. E. Anatomy of the honey bee. Comstock Publishing Company, Ithaca, N. Y. 1956.
11. TOWNSEND, G. F. and LUCAS, C. C. The chemical nature of royal jelly. *Biochem. J.* **34**, 1155-1162 (1940).
12. WEAVER, N. and KUICKEN, K. A. Quantitative analysis of the essential amino acids of royal jelly and some pollens. *J. Econ. Entomol.* **44**, 635-638 (1951).

# THE OCULAR STRUCTURE, RETINOMOTOR AND PHOTO-BEHAVIORAL RESPONSES OF JUVENILE PACIFIC SALMON<sup>1</sup>

M. A. ALI<sup>2</sup>

## Abstract

A histological study of the eyes of juvenile sockeye, coho, pink, and chum salmon in fresh water shows that the cones and external nuclear and plexiform layers of the retinae of embryos and alevins are poorly differentiated and do not attain normal histological or physiological proportions until the emergence of fry from the gravel. From a histophysiological study it is evident that only the emerged fry and older stages are capable of retinomotor responses and that these responses become more marked with age. Differences in rates of adaptation are found among the species and stages. Generally, the pigment layer shows a latent period before contraction in dark. Sensitivity to light is independent of the complete light adaptation of the retinal pigment or visual cells, while full acuity of vision is dependent upon the complete light adaptation of cones. Threshold values of cones and rods are indicated by the feeding and schooling responses. At light intensities between the cone and rod thresholds the thicknesses of pigment and cone layers obey the Weber-Fechner law. There is no diurnal rhythm in the positions of retinal pigment and cones of juvenile *Oncorhynchus* either under constant light or dark. Results are discussed in relation to the migratory, schooling, and feeding behavior. The rapid downstream migration of juvenile salmon during a relatively short period in the night may be related to a semi-dark-adapted state of the eye.

## I. Introduction

The retinomotor responses of the teleost eye are pronounced and several investigations have been carried out to demonstrate the positional changes undergone by the retinal pigment and visual cells in light and dark (2,3,4,14, 18,34,43). Quantitative measurements of the retinal pigment and visual cell layer thicknesses on exposure to different light conditions have been made and presented in the form of graphs by only von Studnitz (42), Kobayashi (30), and Brett and Ali (6).

Visual acuity curves of certain fishes, obtained by using behavioral responses as indices, have been presented by Brünner (7), Wolf and Zerrahn-Wolf (51), Crozier *et al.* (12), Kampa (28), Jones (27), and Yamanouchi (53).

Welsh and Osborn (47), Wigger (50), and Arey and Mundt (5) have observed a diurnal rhythm in the positions of the retinal pigment and visual cells of some fishes exposed to constant light and darkness.

In a preliminary study of the *Oncorhynchus* eye no morphological differences were found in the retinae of the various species (6). This is perhaps surprising since differences have been observed among the species of *Oncorhynchus* in their reactions to light (22,23,24,25,26). It seemed possible that a more

<sup>1</sup>Manuscript received in original form July 28, 1958, and, as revised April 15, 1959.

Contribution from the Institute of Fisheries and Department of Zoology, University of British Columbia, Vancouver 8, B.C.

This research was conducted under the auspices of the Fraser River Hydro and Fisheries Advisory Committee of the University of British Columbia and the results were presented in the form of a thesis for the degree of Doctor of Philosophy of the University of British Columbia. The author wishes to thank Dr. W. A. Clemens, Dr. W. S. Hoar, and Dr. N. J. Berrill for reading and criticizing the manuscript. His thanks are also due to Mr. T. H. Killam for the assistance with draughting.

<sup>2</sup>Present address: Memorial University, St. John's, Nfld.

detailed study of the eye and the ontogeny of photomechanical and behavioral responses of the different species might reveal differences not detected in the initial study. If differences are present these might at least partly explain the somewhat different responses of the various species to light during their downstream migration. This present study is based on the assumption that such a detailed comparative histophysiological examination of the eye would contribute further to an understanding of the mechanisms of downstream migration of salmon.

## II. Material and Methods

### A. MATERIAL

Table I shows the species and stages used in the different experiments and their lengths. All the fish except sockeye and coho smolts were from the hatchery in the Department of Zoology, University of British Columbia.

Maximum light intensities at the surface of the water in the fish troughs were 1.5 to 2.5 ft-c, in January and July respectively. Diurnal light period varied with the season of the year. The water temperature ranged from 7° C to 13° C.

The fish were fed twice daily on a mixture of Clark's dry food,<sup>3</sup> canned salmon, Pabulum,<sup>4</sup> cod liver oil, and yeast extract. The cod liver oil supplement was adequate to satisfy vitamin A requirements essential for the proper functioning of the visual mechanism (14,28).

TABLE I  
Particulars of the material used in this investigation

| Species and stage                    | Length, cm | Experiments in which fish were used |           |         |                            |                   |         | Diurnal rhythm expt. |
|--------------------------------------|------------|-------------------------------------|-----------|---------|----------------------------|-------------------|---------|----------------------|
|                                      |            | Rate of adaptation                  |           |         | Adaptation to l. intensity |                   |         |                      |
|                                      |            | Histo-logical                       | Schooling | Feeding | Histo-logical              | School dispersion | Feeding |                      |
| <i>Sockeye (Oncorhynchus nerka)</i>  |            |                                     |           |         |                            |                   |         |                      |
| Embryo*                              | 0.50       | +                                   |           |         |                            |                   |         |                      |
| Alevin                               | 2.00       | +                                   |           |         | +                          |                   |         | +                    |
| Emerged fry                          | 2.75       | +                                   | +         | +       |                            |                   |         |                      |
| Late fry                             | 3.50       | +                                   |           | +       | +                          | +                 | +       | +                    |
| Smolt                                | 6.80       | +                                   |           | +       | +                          |                   |         | +                    |
| <i>Coho (Oncorhynchus kisutch)</i>   |            |                                     |           |         |                            |                   |         |                      |
| Embryo*                              | 0.80       | +                                   |           |         |                            |                   |         |                      |
| Alevin                               | 2.30       | +                                   |           |         |                            |                   |         |                      |
| Emerged fry                          | 3.70       | +                                   | +         | +       |                            |                   |         |                      |
| Late fry                             | 3.90       | +                                   | +         | +       | +                          | +                 | +       | +                    |
| Smolt                                | 7.10       | +                                   |           | +       | +                          |                   | +       |                      |
| <i>Pink (Oncorhynchus gorbuscha)</i> |            |                                     |           |         |                            |                   |         |                      |
| Embryo*                              | 0.80       | +                                   |           |         |                            |                   |         |                      |
| Alevin                               | 2.40       | +                                   |           |         |                            |                   |         |                      |
| Emerged fry                          | 3.30       | +                                   | +         | +       | +                          | +                 | +       |                      |
| Late fry                             | 3.40       | +                                   | +         | +       |                            |                   |         |                      |
| <i>Chum (Oncorhynchus keta)</i>      |            |                                     |           |         |                            |                   |         |                      |
| Embryo*                              | 0.80       | +                                   |           |         |                            |                   |         |                      |
| Alevin                               | 2.40       | +                                   |           |         | +                          |                   |         | +                    |
| Emerged fry                          | 3.30       | +                                   | +         | +       | +                          | +                 | +       |                      |
| Late fry                             | 3.90       | +                                   | +         | +       | +                          | +                 | +       | +                    |

\*The measurements given for embryos are the diameters of eggs.

<sup>3</sup>Obtained through the courtesy of Mr. J. R. Clark of Salt Lake City, Utah, U.S.A.

<sup>4</sup>Meade Johnson. Prepared mixed cereal.

## B. EXPERIMENTAL METHODS

## 1. General

Experiments were carried out in a light-proof room. Two photometers were used for measuring light intensities. They were a Photovolt Model 200 photometer and a Photovolt Model 520-M electronic photometer. The former was used to measure light intensities above  $10^{-1}$  ft-c and the latter to measure the lower intensities. The model 520-M was used with filters (Eastman Kodak Wratten filters Nos. 81-EF; CC-10-M; 81-C) to produce a curve with equal spectral response to light of wavelengths 3000 Å to 7000 Å. The desired light intensities were created with bulbs of similar spectral ranges. Foot-candle (ft-c) is the unit of light measurement employed throughout. One foot-candle is equal to 10.764 lux (meter-candles).

## 2. Rates of Adaptation

## (a) As Seen by Photomechanical Responses of the Retina

In these experiments animals were sampled at intervals following sudden exposure to bright light after a period in darkness or vice versa. The experiments were carried out using a rectangular galvanized iron trough 137 cm long, 38 cm wide, and 30 cm high, painted black on the inside. Water circulation was maintained during the experiment. The trough was illuminated (400 ft-c) by two Sylvania Reflector flood lamps, fixed on opposite ends of the tank. Diagonal wire frames were placed at the ends of the tank to ensure that all the fish were in the same intensity of light, that is, that none was able to retreat to the less bright corners. The arrangement is shown in Fig. 1.

All the experiments were carried out during the forenoon. Possible variation caused by diurnal rhythm or similar factors was thus minimized. In the light

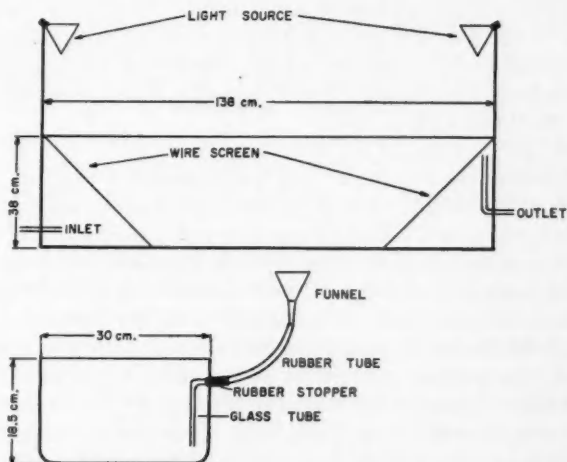


FIG. 1. Diagram showing the experimental arrangement for the various experiments (see text).

adaptation experiments fish were left in total darkness in the experimental tank overnight. At the commencement of the experiment, the first samples were taken in total darkness (zero minutes) and then the lights were turned on and the samples taken after the following times (in minutes): 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, and 70.

In the dark adaptation experiments the fish were left in the illuminated tank (400 ft-c) overnight, the first sample was taken from the illuminated tank (zero minutes); the lights were turned off and samples taken in total darkness at the same intervals as in the case of light adaptation.

The frequency of samples was based on earlier work by Brett and Ali (6) where dark adaptation of sockeye was found to be complete in 50 minutes.

#### (b) *Schooling Times*

The same tank was used. Only emerged and late fry were studied. A group of 50 fish was left in the tank in darkness overnight. The lights were turned on the next morning and the number of schooled fish recorded at 5-minute intervals. If more than two fish were swimming in the same direction, at the same speed, and in a regular formation, this was considered a school. Occasionally two schools were seen in the same tank. In these cases the total number of fish in both schools was taken as the number of fish schooled. Observations were continued until most of the fish schooled or till the strength of the school reached a maximum and the number stayed the same for over 5 minutes.

Some of the recently emerged fry did not form schools within a day or two after emerging. In this case 50 fish were first transferred to another tank under brighter light (400 ft-c), where they schooled readily. They were then used in the experiment after they had had schooling experience.

#### (c) *Feeding Rates*

Fish (six from each group) were first conditioned for at least a week to feed on *Daphnia*. The food was presented each morning in association with a vibratory stimulus given by gently tapping the side of the aquarium with a rod. All learned quickly and after 5 to 7 days of training were able to feed on about 18 to 20 *Daphnia* per minute.

Six aquaria (30 cm long, 24 cm wide, and 24 cm high) were arranged in a large trough of running water with one conditioned fish in each. Each tank had an opening on the side through which a glass tube, inserted through a rubber stopper, was passed. This glass tube reached almost the bottom of the aquarium. The outer end of the glass tube was attached to a rubber tube with a funnel. This arrangement (Fig. 1) was resorted to so that the fish could be fed with very little disturbance. The next morning the lights were turned on (400 ft-c) and 100 *Daphnia* were poured into the aquarium through the funnel after the vibratory stimulus was given. After 5 minutes the fish were quickly removed from the tank and another 100 *Daphnia* poured into the next aquarium in the same way as into the first one after the stimulus was given; after 5 minutes the fish were also removed, and the same procedure repeated in each of the remaining aquaria. After the operation with the sixth tank the water from each aquarium was poured out through a net and the remaining *Daphnia*

in each tank counted. Control experiments without fish in the aquarium showed that the experimental procedure was accurate and no *Daphnia* were lost during the process of pouring through the funnel, capturing, and counting.

### 3. Retinal and Behavioral Responses to Light Intensities

In these experiments the desired light intensities were created by reflecting the light from the white ceiling. The lower light intensities were obtained using a black wooden box with an aperture diaphragm and with a G-E 7.5-watt bulb. The following were the light intensities created:  $10^2$ ,  $10^1$ ,  $10^0$ ,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  ft-c, and almost total darkness. Light intensities were read at different areas of the large trough, and glass aquaria (30 cm long, 24 cm wide, and 24 cm high) were set up in the areas where the light readings were similar.

#### (a) Retinal Photomechanical Responses to Light Intensities

For an hour and a half before they were killed, the fish were left in the light intensity at which they were to be sampled.

With sockeye and chum alevins one group of each was kept under constant light and another batch of each in constant dark for 3 days before they were exposed to different light intensities. This was done to determine whether previous experience had any effect on their reactions to different light intensities. Since no difference was observed this procedure was discontinued in subsequent experiments and fish from the hatchery were directly transferred to the intensity under which they were to be sampled.

#### (b) Feeding Rates under Different Light Intensities

Three fish from each group were conditioned to feed on *Daphnia* as described above. All six types of salmon (Table I) were studied at the same time. The desired intensity of light was set up in the morning and one conditioned fish from each group put in a separate aquarium (30 cm long, 24 cm wide, and 24 cm high) and left under the light intensity for an hour and a half after which the *Daphnia* were fed in the usual manner. After 5 minutes the fish was taken out and the remainder of the *Daphnia* counted. The three fish were thus studied in succession. By adopting this procedure the whole series was completed in 9 days. The remainder of the 100 *Daphnia* were offered to the fish after the experiment to ensure that all fish got the same amount of food each day.

#### (c) Observations on Intensities at Which School Dispersed

Only the late fry were used in this experiment. The experimental arrangement was the same as above. Ten fish were put in the aquarium and left in it for an hour and a half under the light intensity at which they were to be observed to see whether the school was intact or dispersed. The writer was able to observe this without any difficulty after adapting himself (5-10 minutes) to the intensity in question. Under  $10^{-5}$  ft-c, observations were made by suddenly switching on the light and noting whether the school had broken up. Even under the intensities where the animals had formed a school, sudden, bright illumination broke up the school but it was possible immediately after

turning on the light (1-2 seconds) to notice the intact school and then its dispersal. When no school existed the fish were found scattered throughout the aquarium.

#### 4. Photomechanical Responses under Constant Light or Dark

These experiments to test for diurnal rhythm in the positions of the retinal pigment and visual cells in constant light or dark were carried out using the galvanized iron tank described above (Fig. 1). All the experiments were commenced at noon and ended 96 hours later. Animals to be sampled were left in the tank in light (400 ft-c) or in dark and sampled every 3 hours.

#### 5. Histology

For routine study, Bouin's fixative was used, followed by paraffin imbedding and staining with Harris's haematoxylin and eosin. Sections were cut at 8 microns. The whole animal was fixed and after a day its length recorded and the eyes (in the smolts) or the head (in the embryos, alevins, and fry) excised and left in the fixative for another day. The lenses of the late fry and smolts were removed.

To study the neurological arrangement of the retina, a slightly modified version (1) of Golgi's silver impregnation technique was used.

#### 6. Measurement of Retinae and Retinal Layers

In all eyes, thicknesses of retinae, retinal pigment, and cone layers in the dorsal region between the ora serrata and the fundus were measured with a calibrated ocular micrometer. The pigment layer was measured as the distance between the inner border of the choroid and the tips of the pigment projections. The cone layer was measured from the external limiting membrane to the tips of the cone outer segments, since only myoid which is outside the external limiting membrane undergoes elongation and contraction.

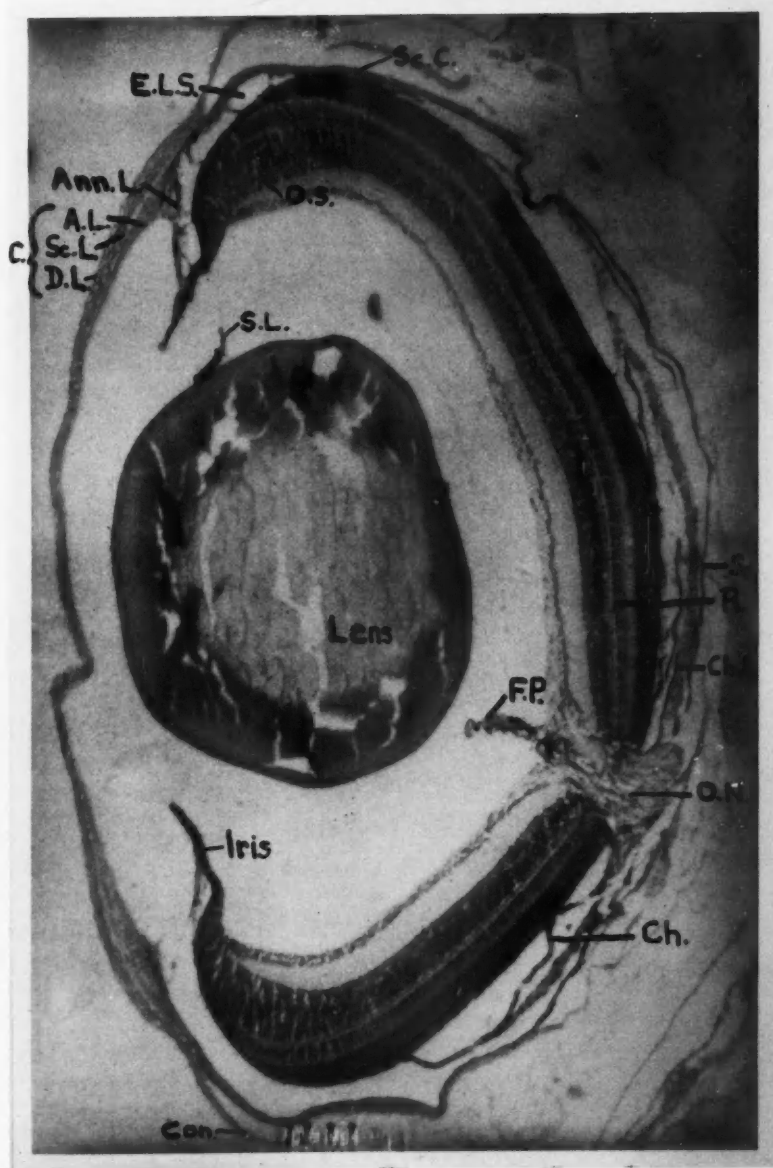
Thicknesses are compared directly, because the examination of nearly 9000 eyes during the course of this investigation suggests that, in a group of fish of the same age and size, the thicknesses of the retinal pigment and cone layers do not vary in direct proportion to the thickness of the retina but do so at random. True, the retina and consequently the various retinal layers are thicker in an older, larger animal than in a younger, smaller one; yet a detailed examination of several fish of various sizes sampled under identical conditions is necessary to establish the relationship between the thicknesses of retinae or retinal layers and the age or length.

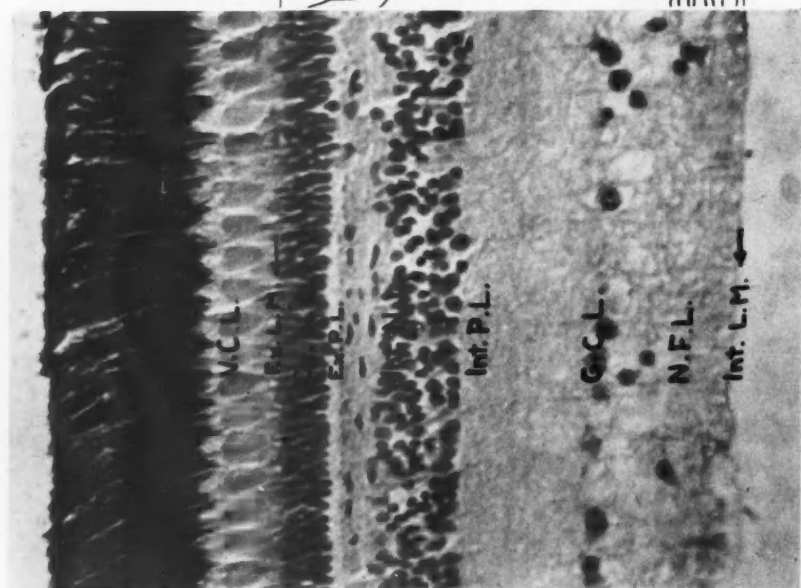
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FIG. 2. Photomicrograph of a vertical section of an *Oncorhynchus* eye.  $\times 90$

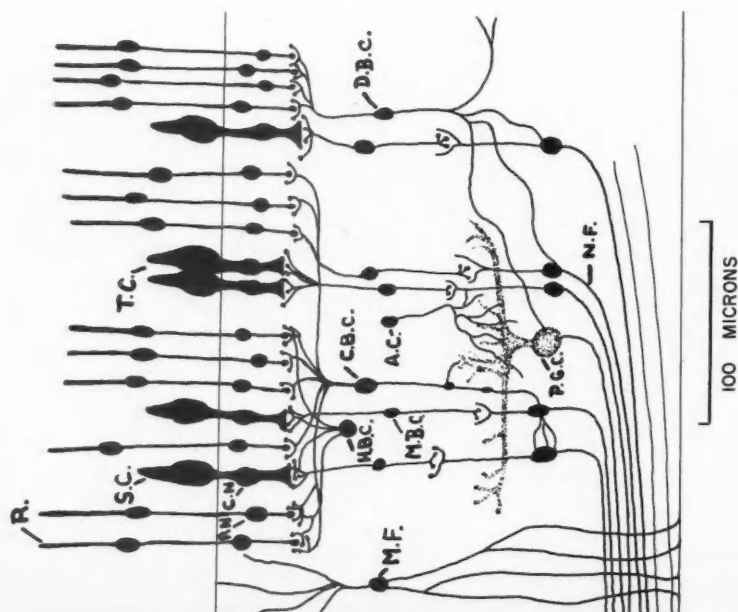
A.L.—Autochthonous layer  
Ann. L.—Annular ligament  
C.—Cornea  
Ch.—Chorioid  
Con.—Conjunctiva  
Ch. G.—Chorioid gland  
D. L.—Dermal layer  
E.L.S.—Epichorioidal lymph space  
F.P.—Falciform process

I—Iris  
O.N.—Optic nerve  
O.S.—Ora serrata  
R.—Retina  
S.L.—Suspensory ligament  
Sc—Sclera  
Sc.C—Scleral cartilage  
Sc.L—Scleral layer





All—Can. J. Zool.



100 MICRONS

The graphs presented consist of points each of which is the mean of 50 measurements made from 10 eyes, except in the case of embryos and alevins when only 20 measurements from four eyes were made.

### III. Results

#### A. STRUCTURE OF THE *Oncorhynchus* EYE (FIGS. 2, 3)

The *Oncorhynchus* eye in its structure follows the typical teleostean pattern and is similar to the eye of the closely related *Salmo* (32, 38, 45, 46). A detailed description of the *Oncorhynchus* eye, including the retina, has already been given in an earlier publication (6) and in the author's thesis (1). The following are a few points of significance.

The iris of the Pacific salmon is not capable of photomechanical changes. Measurements of the iris of intact as well as enucleated eyes exposed to light of low (0.1 ft-c) and high (400 ft-c) intensities did not show any changes in diameter (2 mm).

In the case of embryos, alevins, and emerged and late fry whose whole heads were sectioned, the falciform process appears to encircle a part of the ventral retina. In the dissections of the eyes of juvenile and adult salmon and in the histological sections of the enucleated eyes of late fry and smolts whose lenses had been removed, the falciform process did not present such an appearance (Fig. 2).

No fovea was seen in any of the eyes. The head of the optic nerve (disc) is elliptical and is situated slightly ventral to the center, its orientation being nasotemporal (perpendicular to the longitudinal axis of the eye).

Figure 3 shows a photomicrograph of the Pacific salmon retina together with a diagrammatic presentation of its neurological arrangement seen in Golgi preparations. The neurological arrangement shows greater similarity to that of the primate retina as illustrated by Polyak (36, 37) than to that of the teleostean retina depicted by Franz (17). The "parasol" ganglia have not been described by Franz. Further, he shows a greater number of bipolar cells synapsing with each ganglion cell than is the case in *Oncorhynchus* (Fig. 3).

In addition to the single and twin cones (6) unequal twins were observed during this investigation. These occur rarely in the *Oncorhynchus* retina and

FIG. 3. Photomicrograph of an *Oncorhynchus* retina with a diagram of the neurological arrangement therein.

A.C.—Amacrine cell  
C.B.C.—"Centrifugal" bipolar cell  
C.N.—Cone nucleus  
D.B.C.—"Diffuse" bipolar cell  
Ep. P.L.—Epithelial pigment layer  
Ex. L.M.—External limiting membrane  
Ex. N.L.—External nuclear layer  
Ex. P.L.—External plexiform layer  
G.C.L.—Ganglion cell layer  
H.B.C.—"Horizontal" bipolar cell  
Int. L.M.—Internal limiting membrane  
Int. N.L.—Internal nuclear layer

Int. P.L.—Internal plexiform layer  
M.B.C.—"Midget" bipolar cell  
M.F.—Müllerian fiber  
N.F.—Nerve fiber  
N.F.L.—Nerve fiber layer  
P.G.C.—"Parasol" ganglion cell  
R—Rod  
R.N.—Rod nucleus  
S.C.—Single cone  
T.C.—Twin cone  
V.C.L.—Visual cell layer

are observable more easily in preparations stained longer in eosin. In the region of the fundus, cones are arranged in a mosaic that is similar to that described for *Salmo* by Eigenmann and Schafer (15). However, in the region of the ora serrata they are arranged in rows.

The cones on the dorsal side of the retina are fewer and larger while those on the ventral side are more numerous and more slender. This is pronounced in the emerged and late fry stages in whose retinae the internal nuclear and the ganglion cell layers on the ventral side of the retina are also much thicker since the larger number of cones is accompanied by more numerous "midget" bipolar cells in the internal nuclear layer and correspondingly more ganglion cells. Usually, each cone synapses individually with a single "midget" bipolar, which in turn synapses with one ganglion cell (Fig. 3).

#### B. DIFFERENCES AMONG STAGES

No species differences have been noted in the many Pacific salmon eyes examined. Differences, however, were observed among the stages studied. The following features are of main interest.

The autochthonous layer, the cornea, and the annular ligament are poorly developed in the embryo and recently hatched alevin. They attain maximum development in the late fry stage.

The epithelial pigment of the embryo retina is in a perpetually contracted state (Figs. 16-23) and exposure to any intensity of light for any length of time does not cause the pigment to disperse. The finger-like processes of the epithelial cells, devoid of any pigment, are, however, to be seen. Even in the late alevin stage the pigment migrates only slightly, on illumination. In the older stages such as emerged and late fry, it undergoes dispersion in light and concentration in dark.

Cones are seen clearly in the embryo retina, but their myoids are always fully contracted, presenting the appearance of a constant light-adapted condition. Exposure to dark for any period of time does not cause an expansion. This situation changes somewhat in the alevin stage where cone myoids do possess the capacity to expand slightly in dark. In the older stages the cone myoids show marked expansion in dark and contraction in light.

No rods were found in the embryo retina. Some are seen in the alevin eye. In older stages the rods are clearly visible in the dark-adapted retinae and their myoids are capable of undergoing expansion in light and contraction in dark.

The internal nuclear layer is much thicker (approximately 10 times) than the external nuclear layer in the embryo. The situation changes as the animal grows older. The external nuclear layer thickens with the addition of rods, and in the emerged and late fry the internal nuclear layer is markedly thicker only on the ventral side of the retina due to the greater proportion of cones there. This situation persists, to a certain extent, even in the smolt.

Another difference between the smolt and the younger stages is that the ventral side of the retina in the latter shows less marked photomechanical change than in the former.

The ganglion cell layer of the embryo is also thicker (three cells deep) than that of older stages. With age it decreases in thickness, possibly because the area of the retina also increases. In the late fry it is about one cell deep dorsally and two cells deep ventrally. In the smolts, the ganglion cells are more crowded on the ventral side than on the dorsal side. This, as mentioned elsewhere, is in accordance with the greater proportion of cones.

The molecular layers are very thin in the embryos (Figs. 16-23), and gradually attain the normal proportions with age.

In summary, the embryo possesses all the 10 retinal layers (Figs. 16-23) which the older, fully developed stages possess, but in an entirely different proportion in thicknesses. The proportions gradually change as the animal gets older, eventually reaching a morphologically and physiologically balanced state in the emerged or late fry.

### C. LIGHT ADAPTATION

For want of space, only the adaptation curves of the five stages of coho are presented (Fig. 4). The adaptation curves of the various stages of the other species are quite similar in appearance to those of the coho and may be referred to in the author's thesis (1); however, the times taken by the various stages of the four species studied are presented in the form of a histogram (Fig. 7).

#### 1. Embryos

None of the "dark-adapted" embryos belonging to any of the four species studied showed any retinomotor response on exposure to light (Fig. 4). The pigment remained contracted and the cones that were contracted in the dark remained so (Figs. 16-23).

#### 2. Alevins (*Hatching Stage*) (Fig. 4)

When the dark-adapted alevins are exposed to light, their retinal pigment and cone layers show a slight response as seen in microscopical examination. However, the graphs show no marked changes (Fig. 4). The means of the thicknesses of these layers of animals sampled from zero to 15 minutes after illumination are greater (in the case of the pigment layer) and lower (in the case of the cones) than those of the animals sampled from 20 to 70 minutes, except in the case of the pigment layers of coho and chum alevins indicating that, on the whole, there has been a slight expansion of the pigment layer and contraction of the cone layer on exposure to light. In the case of the pink salmon alevins, no changes were observed even in the histological examination. The most noticeable positional changes of the retinal pigment and cone layers were observed in the case of the coho alevin.

#### 3. Emerged Fry (*No Yolk Sac*)

##### (a) Retinal Response (Figs. 4, 7)

The response of the retinae of dark-adapted emerged fry, on exposure to light, is immediate and in none of the animals did the pigment or cone layers have any measurable latent period before beginning to expand or contract, respectively.

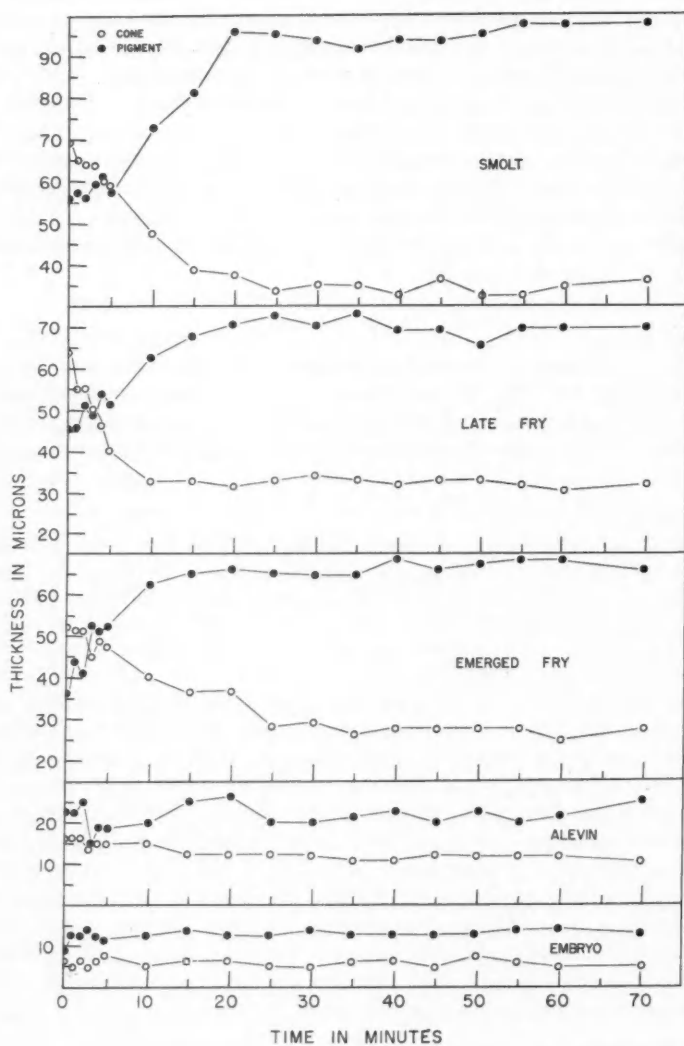


FIG. 4. Graph showing the rate of expansion of the pigment and contraction of cones when dark-adapted coho salmon of various stages are exposed to light of 400 ft-c.

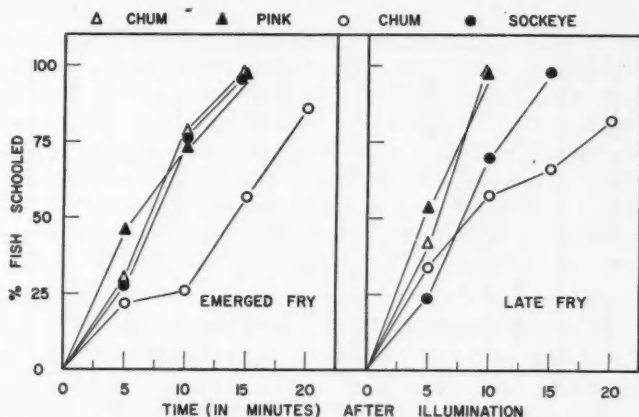


FIG. 5. The rate of schooling of dark-adapted emerged fry and late fry, of the four species, on exposure to light of 400 ft-c.

A point of significance is the difference between the thickness of the fully expanded and fully contracted epithelial pigment of the emerged chum fry. This difference is only 15 microns, as opposed to 25 to 35 microns in the case of the pigment as well as the cones of the other species. Even the cone layer of emerged chum fry shows a difference of 25 microns between the fully expanded and fully contracted cones.

#### (b) Schooling Rates (Fig. 5)

Figure 5 shows the rates of schooling of the emerged fry of all the four species studied, when dark-adapted animals are exposed to light. No correlation between rates of schooling with the adaptation times of either the pigment or the cone layers is evident. In other words, schooling is not dependent upon complete light adaptation. In general, sockeye, pink, and chum each form a school consisting of most of the animals (50) used in the experiment, in about 15 minutes after illumination while the coho takes 5 minutes longer. Another observation that seems pertinent is that unlike the other species, coho school less readily and when they do school, only about 86% of the animals under observation join the school. The others swim around individually. In comparison, the emerged fry of the other species observed (Fig. 5) form schools consisting of 96% or 98% of the animals used in the experiment.

#### (c) Feeding Rates

The feeding rates of conditioned, dark-adapted emerged fry after various periods of illumination are shown in Fig. 6. In every case the maximum rate at which the animals capture their prey occurs only at a time when the cones are also light-adapted as shown by histological examination (compare Figs. 6 and 7). The time at which maximum feeding occurs does not show any correlation with the time taken by the pigment to light adapt fully. The time at which maximum feeding occurred also helped to determine the time for full

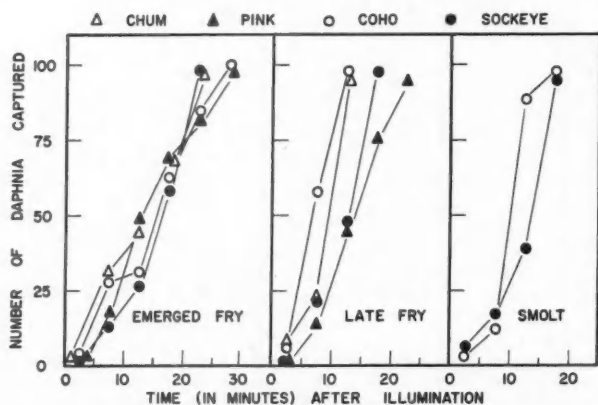


FIG. 6. The rate of feeding of conditioned, dark-adapted, emerged fry, late fry, and smolts of the four species on exposure to light of 400 ft-c.

adaptation of the cones for interspecific comparison. From these experiments it would appear that maximum feeding indicates that the animal's visual acuity is at its best, and this time corresponds well with the time taken by the cones to contract (light adapt) completely, showing the relationship between the cones and visual acuity.

#### 4. Late Fry

##### (a) Retinal Response

The response of the pigment and cones of the dark-adapted late fry of all the four species is immediate and none of them has a measurable latent period (Fig. 4). The pigment of the sockeye late fry is light adapted 10 minutes after illumination while that of the other three species is light adapted in 20 minutes (Fig. 7). The cones of coho and chum light adapt earliest (10 minutes) while those of the sockeye light adapt in 15 minutes followed by those of pink in 20 minutes.

The cones of the late sockeye fry light adapt 5 minutes after the pigment, in contrast with those of the emerged fry and smolts, which light adapt before the pigment does. In the case of the coho, the cones of the late fry light adapt in 10 minutes whereas the cones of the emerged fry take 25 minutes to contract maximally. In the case of the late chum fry, the difference between the fully contracted and the fully expanded pigment layer is 50 microns as compared with the emerged fry where the difference is only 15 microns. The emerged sockeye fry showed differences from the emerged fry of the other species whereas the late sockeye fry did not show any marked differences from the other species. The only aspect in which some difference is seen between late sockeye fry and late fry of other species is the time taken by the pigment to light adapt. The pigment of the late sockeye fry takes the least time to light adapt (20 minutes).

(b) *Schooling Rates* (Fig. 5)

The dark-adapted late fry of sockeye, pink, and chum form schools consisting of 98% of the fish under observation in 10 minutes while the coho take 20 minutes to do so. As in the case of the emerged coho fry, the late fry also do not school readily and when they do, only about 86% of the experimental animals form the school. The time taken to form a school, in the case of the sockeye and late coho fry, corresponds with the time taken by their pigment layers to expand maximally. In the case of the pink no correlation either with the pigment or cones is seen while in the chum the time taken to school is the same as the time taken by the cones to light adapt (10 minutes).

(c) *Feeding Rates*

The coho and chum are able to capture 96% to 98% of the *Daphnia* offered 10 minutes after illumination while the sockeye and pink take longer times (Fig. 6). However, in all cases the time after illumination at which maximum feeding occurred is the same as that taken by the cones to light adapt (compare Figs. 6 and 7). In the case of the pink where the pigment and cones take the same time (20 minutes) to light adapt this correlation extends to the pigment also. As mentioned in the case of the emerged fry here also it is seen that full visual acuity is reached when the cones are light adapted, enabling the capture of maximum number of *Daphnia* possible.

5. *Smolts*

(a) *Retinal Response* (Figs. 4, 7)

The pigment and the cones start expanding and contracting, respectively, immediately after the lights are turned on. The pigment of coho shows a slower movement for the first 5 minutes. In both the species the pigment light adapts in 20 minutes and the cones in 15 minutes (Figs. 6, 7).

(b) *Schooling Rates*

The smolts of sockeye and coho failed to form schools in the experimental tank on exposure to light.<sup>5</sup> A group of 50 fish was observed at 5-minute intervals for half an hour and at 1/2-hour intervals for the next hour and a half, yet it showed no signs of school formation. Occasionally, two or three fish were observed to swim together but this lasted for only a short time after which they separated. Nothing like the school of the emerged and late fry described above was ever observed.

(c) *Feeding Rates*

In both the species maximum feeding occurred 15 minutes after illumination (Fig. 6). Here also, as in the case of the emerged and late fry, it is seen that this time is the same as that taken by the cones to light adapt (Figs. 6, 7). The sockeye smolts take the same time as the late fry to light adapt (15

<sup>5</sup>The emerged fry, late fry, and smolts seemed to be alarmed (?) on illumination and showed something that resembled escape behavior, but this lasted only for a brief period. This alarm reaction seemed more accentuated in the case of the smolts. Among the fry, the sockeye and coho seemed to show a more pronounced reaction than the pink and chum. These observations seem to agree with those of Hoar *et al.* (26). At the time the present experiments were conducted, the author was not aware of the findings of Hoar *et al.* or he would have observed these reactions more closely.

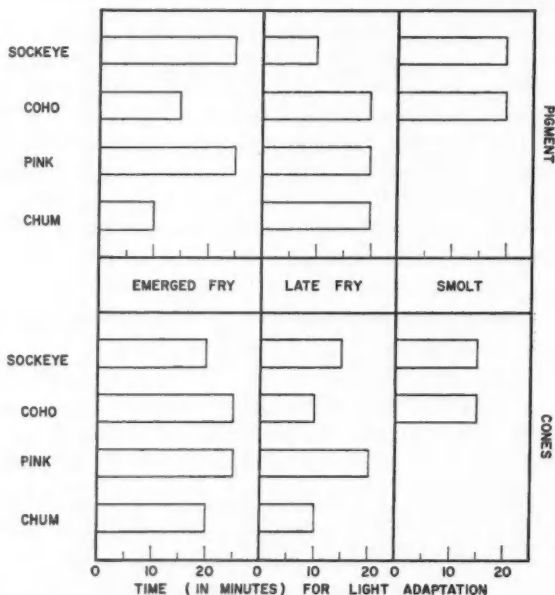


FIG. 7. Histogram showing the times taken by the pigment and cones of dark-adapted emerged fry, late fry, and smolts, of the four species, to light adapt.

minutes), but a longer time than the emerged fry (Fig. 7). The coho smolts, on the other hand, take a longer time than the late fry but a shorter time than the emerged fry (Fig. 7).

#### D. DARK ADAPTATION

In the following pages a description of the process of dark adaptation in the different stages of the four species is given. Here also the results obtained with the five stages of coho are graphically presented. The graphs showing the results with the various stages of the other species are quite similar to these and may be referred to in the author's thesis (1).

##### 1. Embryos (Fig. 4 and Figs. 16-23)

No movement of either the pigment layer or the cones was detected on exposure to dark of a "light-adapted" late embryo.

##### 2. Alevins (*Hatching Stage*)

The thicknesses of the pigment and cone layers of light-adapted alevins sampled at various times after darkening of the room demonstrate no pronounced changes in thicknesses of these layers (Fig. 4). However, examination of the sections of their eyes revealed a slight contraction of the pigment and expansion of the cones in the dark. Also, it is seen that in the alevins of all the four species the means of the thicknesses of the pigment layer and cone

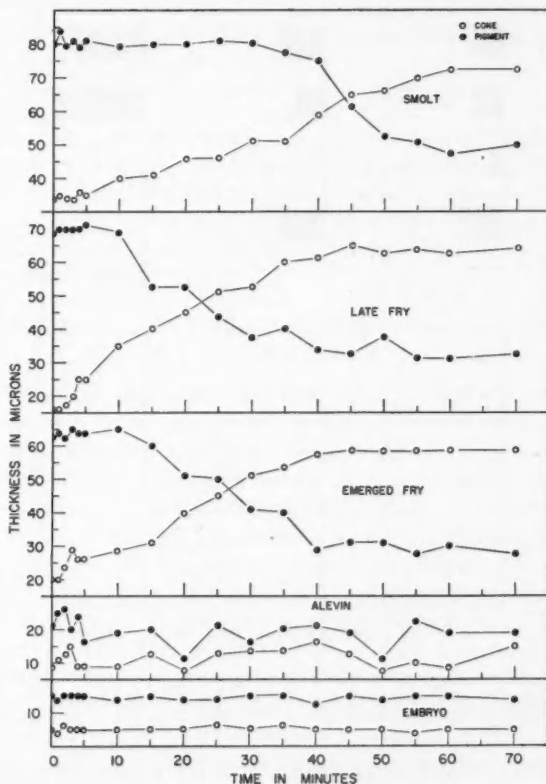


FIG. 8. Graph showing the rate of contraction of pigment and expansion of cones when light-adapted coho salmon of various stages are exposed to dark.

layer sampled from 0 to 15 minutes are consistently higher or lower, respectively, than the means of the thicknesses of these layers of animals fixed from 20 to 70 minutes. The points on the graphs show marked variation, but on the whole, a tendency of the pigment layer to contract and the cones to expand can be observed (Figs. 16-23).

### 3. Emerged Fry (post Yolk Sac)

The pigment of the emerged fry retina shows a latent period before the commencement of contraction in dark. The pink has the shortest (5 minutes) latent period, while the chum has the longest (15 minutes). The sockeye and coho both have a latent period lasting for 10 minutes (Fig. 9). The retinal pigment of the pink takes the longest time to contract maximally (45 minutes). Another point of interest in the case of the pink is that its pigment remains in the half-contracted state for 25 minutes (from 15 to 40 minutes after dark), between the time it begins to contract after the initial latent period, and the time it attains maximal contraction.

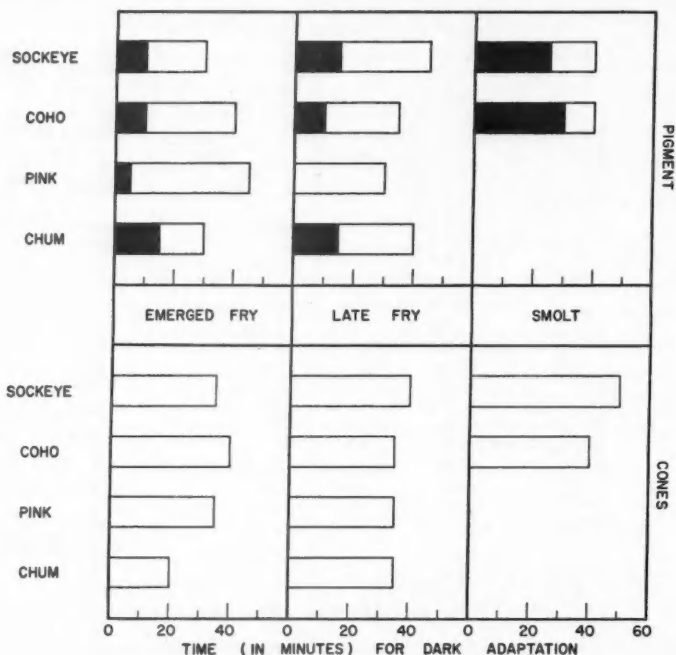


FIG. 9. Histogram showing the times taken by the pigment and cones of light-adapted emerged fry, late fry, and smolts, of the four species, to dark adapt. The solid portions of the bars represent the latent time before the commencement of pigment contraction.

As in the case of light adaptation, the recently emerged chum fry exhibit only a small difference in the thickness between the fully expanded and fully contracted epithelial pigment layer (17 microns). In comparison, the difference between their fully contracted and fully expanded cone layers is 22 microns.

The cones of none of the species have a latent period before commencing migration distally (Fig. 9). The chum cones take the shortest time (20 minutes) and the coho cones take the longest (40 minutes).

#### 4. Late Fry

The epithelial pigment layers of all the species, with the singular exception of the pink, possess a latent period before commencing contraction (Fig. 9). It may be recalled that in the emerged fry stage the pink had a latent period of 5 minutes, which was the shortest as compared with the other species. The sockeye pigment is maximally contracted after 45 minutes, in contrast with the pink whose pigment takes the shortest time (30 minutes) to contract fully.

None of the species shows a latent period before the commencement of the expansion of its cone myoids in dark (Fig. 9). In the case of the cones also,

those of the sockeye take the longest time to expand fully, that is, 40 minutes. The other species take 35 minutes to do so.

#### 5. *Smolts*

The pigment layers of both sockeye and coho possess latent periods, but that of sockeye is somewhat shorter (25 minutes) than that of the coho (30 minutes), but they both take the same time to contract fully (Fig. 9).

The cones of both the species possess no latent period before beginning to expand. The cones of sockeye take a longer time to light-adapt (50 minutes) than those of coho (40 minutes).

### E. RETINOMOTOR AND BEHAVIORAL RESPONSES TO DIFFERENT LIGHT INTENSITIES

The retinal pigment, cones, and rods have different light thresholds. When the light intensity is above the retinal pigment and cone thresholds, the eye assumes the light-adapted state (Fig. 3 and Figs. 16-23). The increase or decrease in light intensity above or below the rod threshold does not bring about any morphological changes in the retina. When the light intensity falls below their threshold values, the retinal pigment and cones contract and expand, respectively, in direct proportion to the logarithm of the light intensity. Generally, they assume a semi-dark-adapted state at certain intensity or intensities below their thresholds. The pigment assumes different stages of semi-contracted state over a wider range of light intensities than do the cones. At some particular light intensity or intensities the pigment and the cones take up positions of complete dark adaptation. The movement of the pigment and cones triggers the simultaneous movement of the rods. It would appear that the pigment, by taking up various positions, regulates the amount of light absorbed inside the optic cup. When it is fully expanded it shields the light-sensitive rods from bright light and exposes them, when it is fully contracted, to light of low intensities. The maximal contraction of the pigment in low light intensities is a device to allow the maximum absorption of the small quantity of light available and its full utilization by the scotopic visual elements, the rods, that are, due to the contraction of their myoids, situated close to the external limiting membrane and directly in the path of the incoming light quanta.

When the eye of the Pacific salmon is adapted for photopic vision the cones are situated near the external limiting membrane (Figs. 2, 3, and 16-23). As long as the light intensity is at or above their threshold they remain there. The feeding rates of the Pacific salmon under the various light intensities used (Figs. 11, 12) show, without any exception, significant correlation between the feeding rates and the state of the cones. As is seen from these experiments, the feeding rate is at its maximum when the cones are in a light-adapted state. When the light intensity falls below the cone threshold and the cones migrate distally, feeding rate also decreases, showing that the visual acuity of the fish is at its best when the cones are in the light-adapted state. This is further supported by the fact that, when dark-adapted, conditioned fish are

exposed to light and their feeding rates recorded at every 5-minute interval after illumination, the time after illumination at which maximum feeding occurs is the same at which the cones also assume fully light-adapted positions (compare Figs. 6 and 7).

When the light intensity falls below the cone threshold, the eyes become adapted for scotopic vision and the rods migrate proximally due to the contraction of their myoids and are situated near the external limiting membrane, the position occupied by the cones in the light-adapted retina. As long as the light intensity is above their threshold, the light-sensitive rods can detect movements and large objects. In the case of the Pacific salmon it would appear that as long as the light intensity is at or above the rod threshold, there is present the ability to see another fish in the same school thus maintaining the school intact. This ability also allows the salmon to detect the prey (*Daphnia*) by locating their silhouette or movement and thus to capture them by visual means. When the light intensity falls below the rod threshold the school disbands and feeding by visual means ceases. The change from photopic to scotopic vision is further indicated by the change in the fish's mode of capturing its prey. As long as the light intensity is above the cone threshold, the animal swims about in the aquarium at all depths, quickly capturing the *Daphnia* that it sees, swallowing one and locating another simultaneously, and feeding rate is at its highest (19 to 20 per minute). When the light intensity decreases below the cone threshold, the shiftover to scotopic vision occurs and the animal resorts to an altogether different mode of capturing its prey. It stays in the bottom third of the tank, its body at a slight angle with the bottom, locates the prey by its movement and silhouette, then makes a dash upward, captures it, and returns to the bottom of the tank. The rate at which it captures the prey depends on the light intensity, for, as the intensity declines more and more there is greater difficulty in detecting any movement or silhouette, hence the decrease in the feeding rates under lower light intensities. If any feeding occurs when the light intensity is below the rod threshold it is due to the employment of some other sensory perception. In the case of the Pacific salmon, the rate of capture of *Daphnia* in a completely dark room found to be often zero and rarely one or two in 5 minutes.

The feeding rates under light intensities between the rod and cone thresholds obey the Weber-Fechner law (Figs. 10, 11, 12).

In the following pages, there will be described the retinal and behavioral responses, of the species and stages used, to different light intensities. The responses of *Oncorhynchus* to various light intensities are summarized diagrammatically (Fig. 14) together with values of various light intensities in nature.

#### 1. Alevins (2 or 3 Weeks after Hatching) (Fig. 10)

The alevins used were not newly hatched as were those in the adaptation experiments but about two or more weeks older, some almost approaching the emerging stage. These showed notable changes of visual cell layers under different light conditions.

No differences were observed in the reactions of the retinal pigment and cones to different light intensities between the alevins that were kept in darkness and those that were kept in light, for 3 days, prior to the experiment (Fig. 10). The pigment of the sockeye was fully expanded until the intensity fell below  $10^0$  ft-c, and was fully contracted at intensities below  $10^{-2}$  ft-c. The chum pigment seemed to have a lower threshold for the commencement of contraction ( $10^{-1}$  ft-c), and was seen to be fully contracted at intensities of  $10^{-2}$  ft-c, or lower.

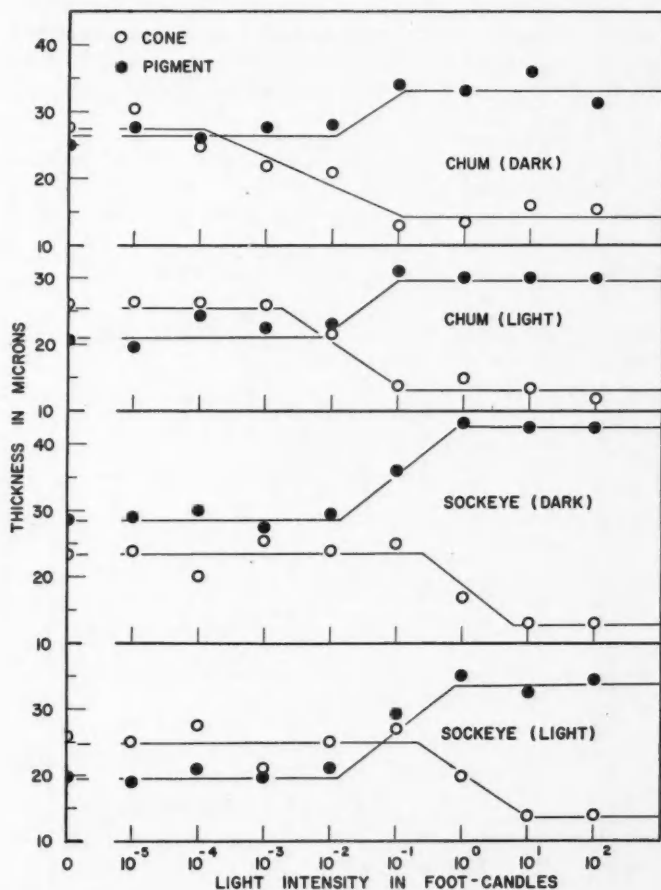


FIG. 10. Graph showing the thicknesses of pigment and cones of sockeye and chum alevins in various light intensities. (Light) and (dark) refer to the fish that were kept under light or dark, respectively, for 3 days before exposure to the various light intensities mentioned.

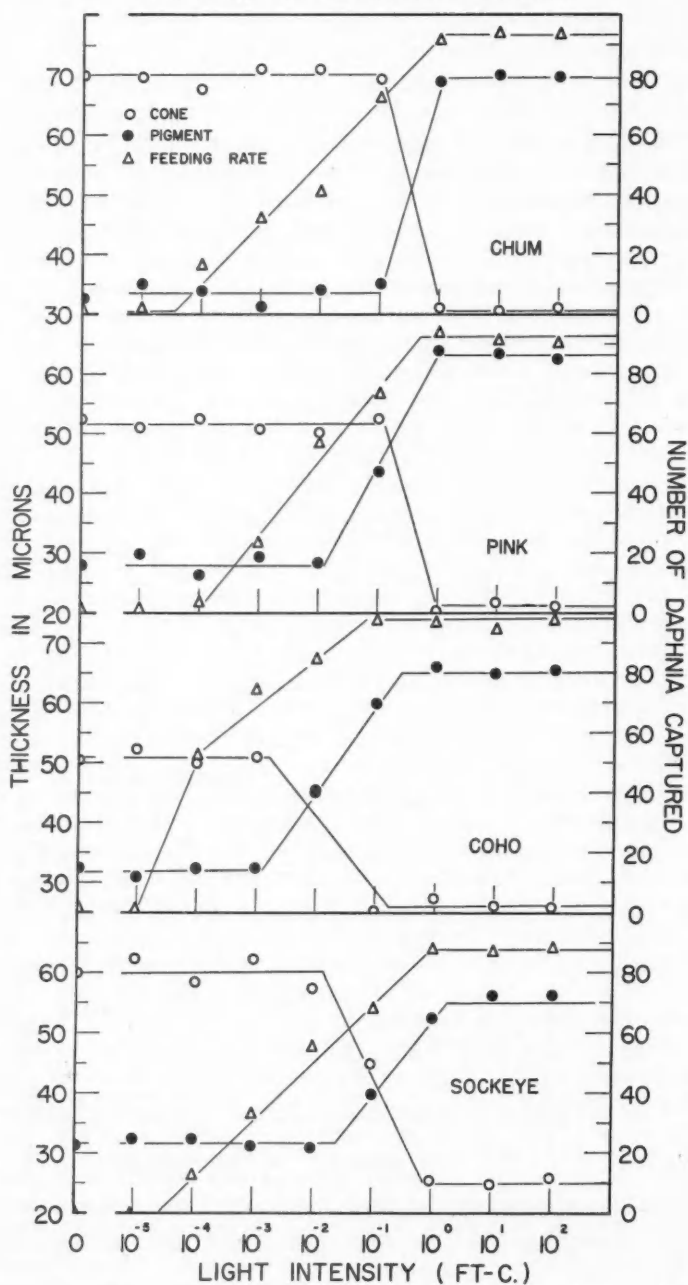


FIG. 11. Graph showing the thicknesses of pigment and cones and the feeding rates of late fry of the four species under various light intensities.

The cones of both sockeye and chum were maximally expanded until the intensity decreased below  $10^{-2}$  ft-c. The cones of the former were fully contracted at  $10^{-1}$  ft-c, or lower, while those of the latter did not do so until the intensity fell below  $10^{-3}$  ft-c, or lower.

## 2. Late Fry (Figs. 11, 13)

The pigment layer of all species, except sockeye, remains fully expanded until the intensity of light falls below  $10^0$  ft-c. That of sockeye starts contracting when the intensity falls below  $10^1$  ft-c. Maximal contraction of the pigment occurs at various intensities in the different species (Fig. 11). Here the two extremes seem to be coho ( $10^{-3}$  ft-c) and chum ( $10^{-1}$  ft-c).

The cones of all species except coho do not start expanding (dark-adapting) unless the light intensity decreases below  $10^0$  ft-c (cone threshold). This

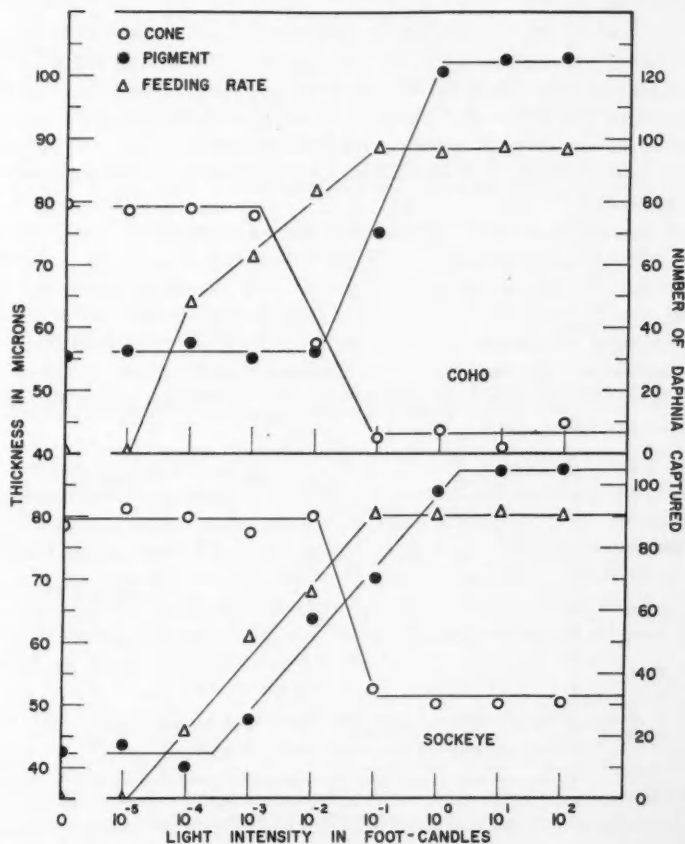


FIG. 12. Graph showing the thicknesses of pigment and cones and the feeding rates of sockeye and coho smolts under various light intensities.

intensity is lower ( $10^{-1}$  ft-c) in the case of coho (Fig. 13). The cones are maximally expanded at  $10^{-1}$  ft-c, in the case of pink and chum, while in the sockeye this occurs at  $10^{-2}$  ft-c, and at  $10^{-3}$  ft-c in the case of the coho.

The feeding rates under the different light intensities studied show a correlation with the state of adaptation of the cones (Fig. 11). Changes in feeding behavior, when the light intensity falls below the cone threshold, are similar in all the species. When vision changes from photopic to scotopic, the animals stay in the bottom third of the tank and capture their prey by detecting their movements and silhouettes. It appears from these experiments that the cones of coho have the lowest threshold ( $10^{-1}$  ft-c), while the others have a higher threshold ( $10^0$  ft-c).

No feeding occurs at intensities of  $10^{-5}$  ft-c or lower. The rods of all the species studied have the same threshold ( $10^{-4}$  ft-c).

### 3. Smolts (Figs. 12, 13)

The pigment of the sockeye smolt, like that of the sockeye late fry, does not commence contracting until the light intensity decreases below  $10^1$  ft-c, which is higher than the minimum intensity at which the coho pigment is fully expanded ( $10^0$  ft-c). As in sockeye, the coho smolts also show similarity with the fry. The retinal pigment layer of sockeye is maximally contracted at a lower light intensity ( $10^{-4}$  ft-c) than that at which the coho pigment maximally contracts ( $10^{-2}$  ft-c).

The cones of both sockeye and coho smolts do not commence their migration distally unless the light intensity falls below  $10^{-1}$  ft-c (Figs. 12, 13). However, the maximum intensities of light at which they are fully expanded (dark-adapted) are different. They are  $10^{-2}$  ft-c (sockeye) and  $10^{-3}$  ft-c (coho).

In the case of the smolts also, feeding rates show agreement with the state of adaptation of the cones (Fig. 12). The change in the mode of capture of the prey when light intensity falls below the cone threshold is also the same as in the fry, described above.

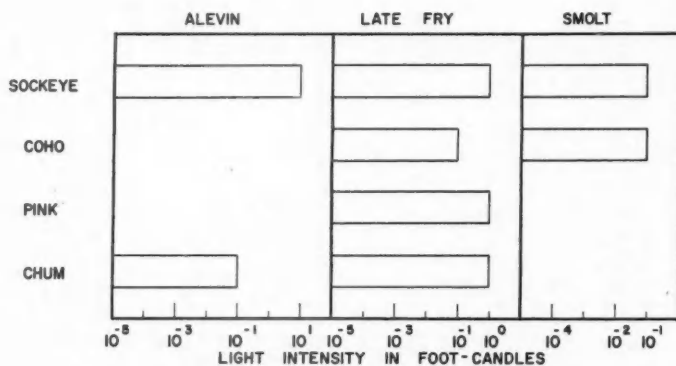


FIG. 13. Histogram showing the cone thresholds of the various stages of the four species. Note that the late coho fry has a lower threshold.

The cone ( $10^{-1}$  ft-c) and rod ( $10^{-4}$  ft-c) thresholds, the intensity at which feeding by visual means stops ( $10^{-5}$  ft-c) are the same in both the sockeye and coho smolts (Figs. 12, 13).

#### 4. General Observations

In all cases, the pigment and cone layers at light intensities lower than their thresholds showed semi-contracted and semi-expanded stages respectively, with the exception of the pigment and cone layers of the late chum fry, the cones of the pink fry and sockeye smolts. This is perhaps understandable in the case of the pigment layer for its function seems to be to control, by expansion or contraction, the amount of light absorbed inside the optic cup, but one would assume that the cones would stay light adapted as long as the light intensity exceeds their thresholds and then when it decreases below the thresholds, they would dark adapt. These intermediate stages seen in the

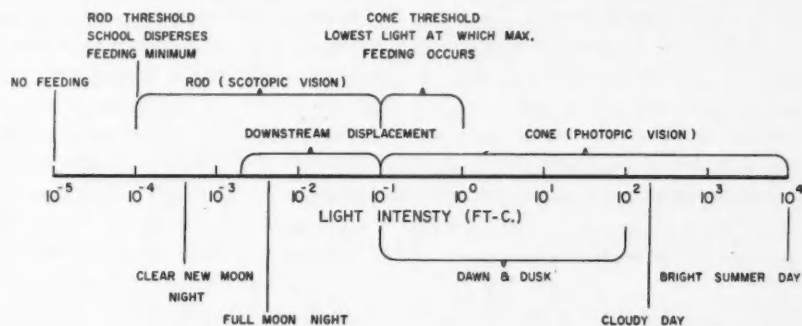


FIG. 14. Diagram summarizing the results obtained under various light intensities together with some other responses of *Oncorhynchus* to different light intensities. Light intensities under natural conditions are also indicated for comparison.

case of cones perhaps suggest that they are not altogether useless at light intensities below their thresholds. The absence of intermediate stages of contraction and expansion of pigment and cones respectively in the exceptions mentioned above may be due to the reason that the intermediate states occur at some light intensity or intensities between the two intensities at which they were fully light-adapted and dark-adapted. On the other hand, this may not be the case, but may be characteristic of these particular stages. Further investigation alone can answer this.

#### F. STATE OF THE RETINAL PIGMENT AND CONE LAYERS UNDER CONSTANT LIGHT OR DARK

If the retinal pigment and the visual cells were to exhibit a diurnal rhythm, they would neither be completely dark-adapted during day when kept under constant dark nor would they be completely light-adapted during night when kept under constant light. Diurnal rhythm, when present, is apparent only

in constant dark or is less pronounced in constant light (5, 47, 50). In instances where diurnal rhythm is present it is more marked on the first day, less so on the second, eventually disappearing altogether. The period of its persistence varies.

The retina of an animal that does not possess a diurnal rhythm will be fully light or dark adapted under constant light or dark, respectively, without regard to the time of day.

In the species and stages that were used in these experiments (Table I), no differences were observed between the positions taken by the retinal pigment of the animals fixed during the day and those fixed during the night irrespective of whether they were under constant light or constant dark.

In all the histological preparations the retinae of animals subjected to constant light appeared fully light-adapted whether they were sampled during the day or during the night. Likewise, the retinae of animals kept in darkness were fully dark-adapted irrespective of the time of the day at which they were sampled. It does not appear that the pigment or the cones have a diurnal rhythm or indeed any rhythm.

However, when the thicknesses were plotted against the time the animals were sampled, it was seen that except in a few cases the points of the graph did not present the appearance of lying in a straight line as is seen, for example,

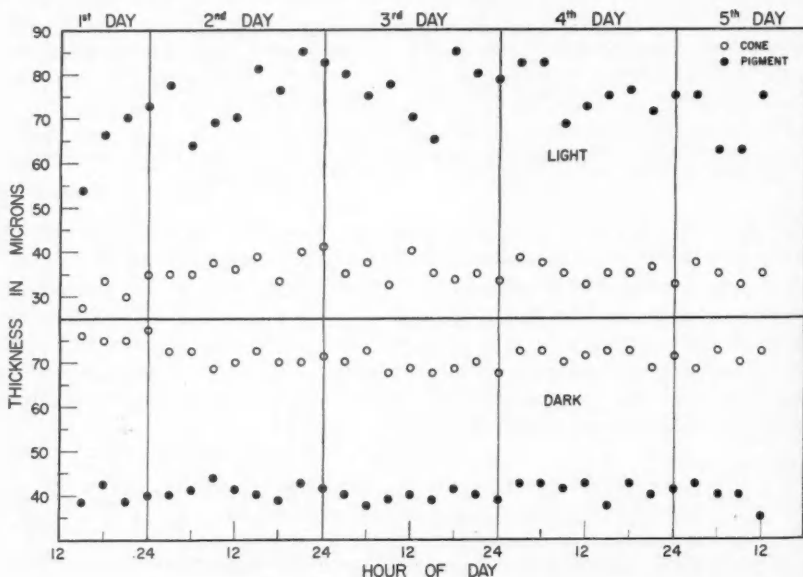


FIG. 15. Graph showing the thicknesses of pigment and cones of late chum fry at 3-hour intervals for 96 hours, under constant dark are given as an example where no variation is seen and the data under constant light are given to show an extreme case of variation in thicknesses without any diurnal rhythm being seen.

in the case of chum fry in dark, whose data are presented (Fig. 15). In most cases, although the pigment layer was fully light-adapted or dark-adapted, depending on whether the animal was held in light or dark, its thickness showed marked variation. This was less frequent in the case of the cones. The data for chum fry in light are presented (Fig. 15) as an example of an extreme case of variation. In addition, it also shows a tendency of the pigment layer to be slightly expanded in the nights of first 2 days. The reason for this, perhaps, is the building up of acid as has been described by von Studnitz (42) and Wigger (49, 50) in the case of the gold fish. On the other hand, it might be that constant light is a stress and as such disturbs the physiological balance in the animal's metabolism. Once the physiological balance is altered or upset, the changes in the secretion of hormones that control phenomena such as pigment dispersion or contraction could bring about changes in the thickness or position of pigment which are not diurnal but random, depending on the effect of endocrines. It has been found that intermedin regulates the migration of retinal pigment in fishes (35). In all other cases whether in constant light or constant darkness, the conditions varied between the two extreme conditions shown in Fig. 15. In general, the variation is greater in older animals such as smolts, most probably due to the greater thickness of their retinae.

Statistical analyses were carried out and no significant differences could be established either between the day data and night data or between the midnight data and the midday data or between the thicknesses of these layers of animals sampled during the first 2 days and those sampled during the last 2 days (3rd and 4th days).

It seems reasonable to conclude that in *Oncorhynchus* there is no diurnal rhythm in the positions of retinal pigment and cone layers and that the variations in the thicknesses of the pigment layer may be related to the action of intermedin.

#### IV. Discussion

##### A. STRUCTURE OF THE *Oncorhynchus* EYE

The eye of the Pacific salmon possesses features such as the sclera, cornea, lens, iris, and an inverted duplex retina with all the 10 layers that are characteristic of a typical vertebrate eye, including the human. However, since it is a teleost eye, it shows certain structural and functional features that are peculiar to most teleosts. These can be listed as the cup-shaped, oval eye with its flattened anterior surface, proportionately larger cornea, spherical lens, retractor lentis, non-contractile iris, a "supplementary nutritive device" consisting of a falciform process, chorioid gland, accommodation (for distant or near vision) by shifting of the lens axis, the presence of twin cones, and the capacity of the visual cells and retinal pigment to undergo photomechanical changes.

## B. CORRELATION BETWEEN THE ARHYTHMIC MODE OF LIFE OF *Oncorhynchus* AND THE STRUCTURE OF ITS RETINA

The heavily pigmented epithelial layer, three types of cones, and the abundance of rods (Fig. 3), together with the ability of these visual elements to undergo remarkable photomechanical changes, suggest that the Pacific salmon is what Walls (46) would call "arhythmic".

This is certainly borne out by the results obtained in this investigation. It has been shown (Figs. 11, 12, 14) that the juvenile Pacific salmon are capable of carrying on activities such as feeding and schooling under widely different light conditions.

The presence of three types of cones and the complex neurological arrangement of the retina suggest that the Pacific salmon might be capable of color vision.

## C. RETINOMOTOR (RETINAL PHOTOMECHANICAL) RESPONSES

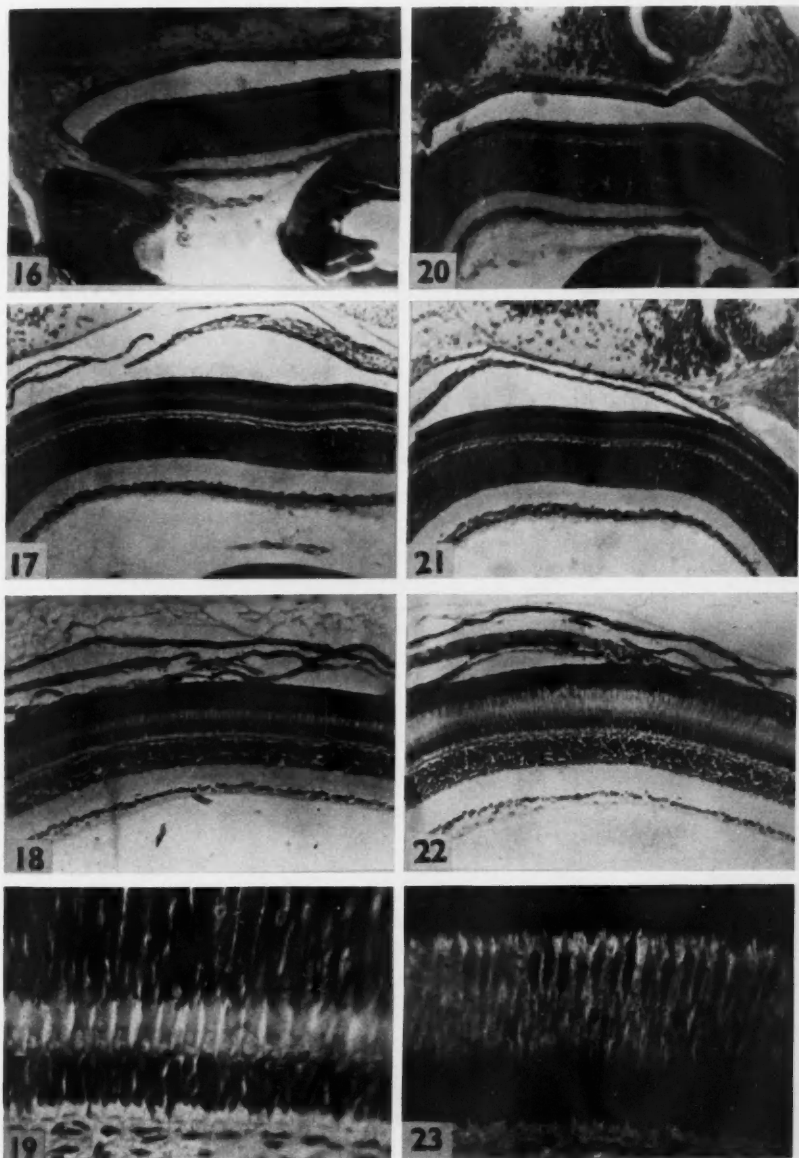
### 1. *Ontogeny of Retinomotor Responses in Oncorhynchus*

As has been shown in this paper (Figs. 4, 8, and Figs. 16-23), retinal photomechanical changes occur neither in the embryos prior to hatching nor in the newly hatched alevins of any of the four *Oncorhynchus* species studied. The situation changes as the alevins become older. Two or three weeks after hatching, the alevin with a comparatively small yolk sac shows retinomotor responses to different light conditions (Fig. 10). In the emerged fry, the ability of the retina to undergo extensive photomechanical changes (Figs. 4, 8, and 16-23) and emergence from the gravel, with the consequent exposure to different light conditions, coincide. This ability is more marked in the late fry and is perfected in the smolts (Figs. 16-23).

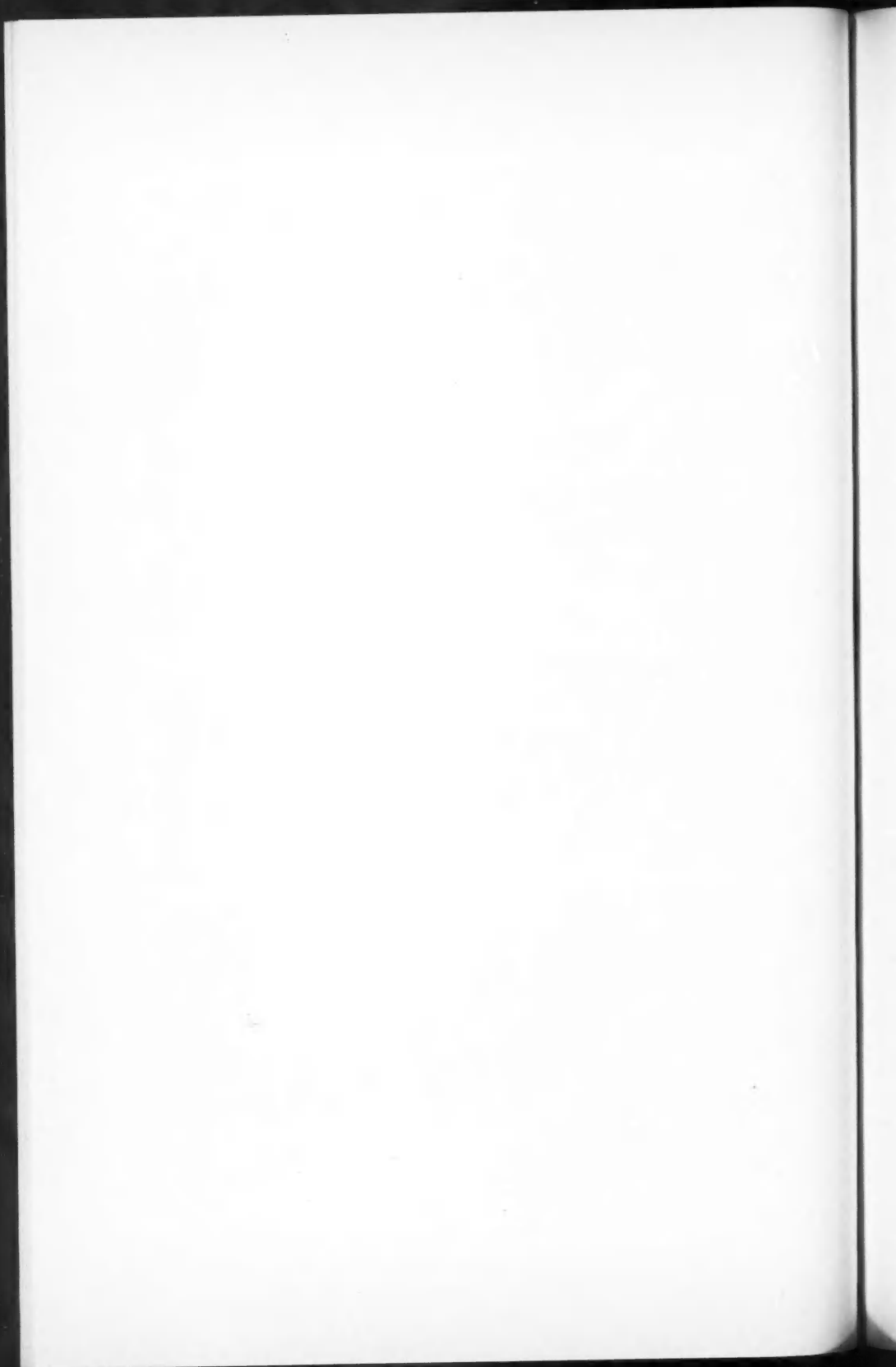
Since no study of the ontogeny of photomechanical responses in other teleosts or in any other vertebrate group is available, it is not possible to state with certainty whether this situation is common to all teleosts or to other vertebrates whose retinæ are capable of undergoing photomechanical changes.

### 2. *Interspecific Comparison of Retinomotor Responses of the Species and Stages of Oncorhynchus*

Clemens (11) wrote, "On the basis of morphological, physiological, life-history and behavior studies to date, it appears that spring and coho salmon are related on the one hand, pink and chum on the other and that sockeye occupy a position more or less intermediate between the two pairs". After several years of study of the behavior of juvenile *Oncorhynchus*, Hoar (25) has come to the conclusion that the coho is the closest to the parental, trout-like type, while the pink and chum are the most specialized. Specific differences have been found among the juvenile *Oncorhynchus* in their reactions to light (26). However, no differences in their ocular structure were found in this as well as an earlier investigation (6). Hence, it was of interest to note whether they showed any interspecific differences in their retinomotor responses.



FIGS. 16, 17, 18, 19, 20, 21, 22, and 23. Photomicrographs of light-adapted and dark-adapted retinæ of various stages of Coho salmon. Figures 16 to 19 are light-adapted retinæ of late embryo, alevin, emerged fry, and smolt respectively. 20 to 23 are dark-adapted retinæ of late embryo, alevin, emerged fry, and smolt respectively.



As has been shown in the results, the various species and stages studied did show differences in their rates of light or dark adaptation and retinal responses to different light intensities. However, these differences are not consistent (Figs. 7, 9, 13) and do not appear to indicate interspecific relationships.

#### D. SIGNIFICANCE OF RETINOMOTOR RESPONSES IN THE LIFE OF JUVENILE *Oncorhynchus*

##### 1. Precedence of Light Sensitivity to Retinomotor Responses

It has been shown that the eyes of newly hatched alevins are not capable of undergoing photomechanical responses. However, they are photosensitive as seen by their negative response to light. Hoar (25) considers the tendency to hide under stones (as a result of photonegative response) to be their complete behavior at this stage. The *Oncorhynchus* alevins become less photonegative and increasingly photopositive with age. This coincides with the greater development of the retinal elements which are capable of responding to light (Figs. 10 and 16-23). This culminates in the photopositive emerged fry that shows marked photomechanical changes (Figs. 4, 8, and 16-23), and also possesses full visual acuity as shown by the feeding experiments (Fig. 6).

The newly hatched demersal alevins of fresh-water fish are known to be photonegative (8, 19, 21, 39, 41, 48, 52). It is not known whether the retinae of these alevins are capable of undergoing photomechanical changes or not. In contrast to the demersal alevins of the fresh-water species, the pelagic larvae of marine fish that have been studied so far are all reported to be photopositive (9, 13, 16, 40, 44). Again, it is not known whether their eyes show retinomotor responses.

##### 2. Migratory Behavior

As Clemens (10) remarked, "The migration of Pacific salmon is another excellent illustration of the delicate interrelations between organisms and the environment; in other words, of the interplay between a physico-chemical organism and a physico-chemical environment". The downstream migration, which takes place at dusk, is a combination of the fish's response to light and its individual behavior pattern (23). It has been shown that the eyes of sockeye and coho smolts and also those of pink and chum fry are in the process of dark adapting at the time of the commencement of downstream migration (6).

Hoar (23, 25), Neave (33), and McDonald (31) have shown that as the light intensity decreases at dusk, the fry of the migrating species (sockeye, pink, and chum) rise to the surface and either swim with the current or are displaced. The mechanism of downstream migration is similar in fry and smolts. The coho fry do not show the same marked increase in activity as the other species at dusk. Due to this, they ordinarily do not rise to the surface and become displaced at dusk. They are, however, subject to some displacement, particularly in times of high water (25). The fact that the cone threshold of coho fry ( $10^{-1}$  ft-c) is lower than that of the other species (Fig. 13), while their

rate of dark adaptation is very similar (Fig. 9), might be partly or wholly responsible for this difference in their behavior. They will, in short, be able to see at lower light intensities.

Evidence has been presented in the present investigation to show that when the light intensity falls below the cone thresholds ( $10^{-1}$  to  $10^0$  ft-c) the eyes of fry and smolts commence to dark-adapt (Figs. 11, 12). It has also been shown that the process of dark adaptation takes ordinarily (with the exception of emerged chum fry) 35 to 40 minutes in the case of fry and 40 to 50 minutes in the case of smolts (Fig. 9). In the face of this evidence, it is suggested that these fish commence migration as the light intensity begins to decrease rapidly and falls below the cone threshold. This may result in a state of partial night blindness. At this stage the rate of decrease of light intensity in nature is very rapid and decreases from 1.0 ft-c to 0.002 ft-c in 30 minutes (6). Its rate is greater than the rate of dark adaptation as found in this investigation. This leaves the animal in a semi-dark-adapted state which results in its losing its ability to maintain position with relation to some reference point and it swims with the current, or is displaced downstream.

Since the process of dark adaptation takes 35 to 40 minutes for completion in the fry, those that have risen to the surface at the time of dusk in a semi-adapted state for 35 to 40 minutes consequently swim with the current or get displaced during this entire period. When the process of dark adaptation is completed the light intensity at the surface of the water is well above the rod threshold ( $10^{-4}$  ft-c) and the fry are able to see large objects such as rocks and use them as reference points and migration ceases or slows down considerably. This suggests that the states of adaptation of the eyes are responsible for the marked peak in the downstream migration of the juveniles at dusk. This peak in migration lasts for a longer time in the case of sockeye smolts (Dr. W. A. Clemens, personal communication). This appears to be due to the process of dark adaptation taking a longer time (50 minutes) in the case of sockeye smolts (Fig. 9).

This peak in the downstream migration of the *Oncorhynchus* juveniles may have survival value, especially in the case of smaller fry. As Hoar (25) remarks, "When many small fish must face a fixed number of predators, the shorter and more precise the period of contact, the better will be their chance of survival". The slow rate of dark adaptation coupled with the rapid decrease in light intensity triggers their mass migration lasting for a brief period, with its obvious advantages.

Another point that warrants mention here is the fact that these migrations at dusk are related, not to the time of the day, but to the light intensity (31). In other words, as the summer day gets longer and longer, the commencement of migration shifts to later times in the evening corresponding to the intensity of light. The absence of diurnal rhythm in the positions of pigment and cones, as shown in this investigation (Fig. 15), makes possible this response to light intensity, for, if there were a sharp diurnal rhythm in the positions of the visual cells and pigment layer, the animal would possess an eye in a particular

state of adaptation irrespective of the intensity of light available. This would result in its coming up to the surface and swimming or being displaced with the current at the same time every day.

It would appear that in the guidance of juvenile downstream migrants around barriers such as dams by visual stimuli (e.g. illuminated moving screens), the fact that the eye of the migrating fish is in a semi- or fully dark-adapted state (6), and that it takes 10–20 minutes for it to light-adapt fully (Fig. 7), may be important. Unless the eye is in a light-adapted state, visual acuity, so necessary for the animal to be able to follow the moving screen, is not at its best as shown by the feeding experiments conducted in the present investigation (Fig. 5; also compare Fig. 7).

### 3. Schooling

That sight is the primary requisite in the formation and maintenance of fish schools has been established (25, 29), and this investigation presents more evidence to support this. It has been shown in the results that schools of juvenile *Oncorhynchus* disperse in total darkness and in light intensities lower than the rod threshold ( $10^{-4}$  ft-c). Pink, chum, and sockeye fry, which are known to school readily (25), form schools 10 to 15 minutes after dark-adapted fish are exposed to light (Fig. 5). The coho, on the other hand, school less readily as shown by the longer time they take to school and the fewer animals that participate in this process (Fig. 5). The time taken by the dark-adapted animals, on exposure to light, to school is less than the time taken for full light adaptation (compare Figs. 5 and 7). From this it would appear that full acuity of vision is not necessary for a fish to recognize and join another fish of the same species to form a school. This is supported by the fact that schools persist in dim light when the eyes are adapted for scotopic vision. Once formed, a school stays intact (other conditions remaining the same) until the light intensity falls below the threshold for rods, when even the shapes of large objects are not recognizable.

### 4. Feeding

As Hoar (25) observed, "The Pacific salmon is basically a surface feeding fish, depending on its eyes for the location and capture of its food". This has been well exemplified by the results obtained in this investigation. Active feeding stops in the dark, save for the occasional chance capture of prey. In feeding experiments, in which live prey are used, the behavior of the prey under different light intensities will affect the results significantly. However, in the case of *Daphnia*, it has been shown (20) and also noticed during the course of the present investigation that on exposure to total darkness, the *Daphnia* will sink to the bottom only after 10 to 15 minutes, whereas experiments in this investigation did not last over 5 minutes.

It has been shown that feeding does not occur when the light intensity is lower than the rod threshold (Figs. 11, 12). It occurs at higher intensities but between the rod and cone thresholds (scotopic vision) is proportional to the logarithm of the light intensity (Weber-Fechner law). When light intensity

increases to cone threshold or higher, it reaches its maximum. Whether very high intensities reduce feeding rates is a matter of conjecture but intensities as high as  $63 \times 10^2$  ft-c did not affect maximum feeding in coho fry (unpublished data).

The change in the mode of capture of the prey as the intensity falls below the cone threshold is interesting and indicates the ability of the rods to resolve as the cones do. That the visual acuity is lost in the scotopic visual field of the animal is shown by the change to the "silhouette method" of feeding whereby the animal makes use of the sensitivity of rods and detects the shadow of the prey against the brighter background. With decreasing light intensity the difference between the shadow and background diminishes, making the location of prey more and more difficult. This accounts for the reduction in feeding rates, in proportion to the logarithm of the light intensity (Figs. 11, 12). When the light intensity falls below the rod threshold, the shadow of the prey cannot be distinguished from the background by the animal, resulting in its inability to locate the prey, and feeding stops.

### V. Summary and Conclusions

1. The ocular structure and retinal responses of late embryos, alevins, emerged fry, late fry, and smolts of four species of *Oncorhynchus* to various light conditions were studied and correlated with their schooling, feeding, and migratory behavior. Experiments were also conducted to ascertain whether their retinal elements exhibited any diurnal rhythm in their positions when kept under constant light or dark.

2. The structure of the *Oncorhynchus* eye is typical of vertebrate eyes in general and teleost eyes in particular. It possesses an immobile iris and a chorioid gland and a falciform process. The neurological arrangement of the retina is similar to that of the primates.

3. The eyes of the late embryos are not fully developed histologically and physiologically and are not capable of retinomotor responses. The eyes of the alevins are also not fully developed but show some response to light. Their ability to undergo photomechanical changes increases with age.

4. As the animals become older they show a general trend in shortening of the time required for light adaptation. The time taken for dark adaptation on the other hand, shows a tendency to increase with age. Dark adaptation takes a longer time than does light adaptation.

5. The retinal epithelial pigment layers of all the fish studied, except that of the late pink fry, have a latent period before commencement of contraction in the dark. The latent period is longer in older sockeye and coho. It does not change with age in the chum salmon. In the case of pink salmon it is very short (5 minutes) in the emerged fry and is absent in the late fry.

6. The four species of Pacific salmon studied show differences in their retinal and behavioral responses to light. In all cases, the state of the cones and the ability to capture maximum number of *Daphnia* are correlated and indicate the time of complete light adaptation and cone thresholds.

7. The sockeye show a lowering of the cone thresholds with age; in the coho there are no differences among the stages, while the chum fry have a higher cone threshold than do the alevins. Only the fry of pink were studied. The rod threshold ( $10^{-4}$  ft-c) is the same in all species and stages. The feeding rates and thicknesses of the pigment and cone layers obey the Weber-Fechner law in the intensities between the rod and cone thresholds.

8. Under constant light or constant dark, there is no diurnal rhythm in the positions of the pigment and cone layers of the Pacific salmon.

9. Based on this research it is suggested that the downstream migration of juvenile Pacific salmon occurs as a result of their eyes being in a semi-dark-adapted state for a short period at dusk. This happens due to the rapid decrease in the incident light intensity and the relatively slower rate of dark adaptation. The fish lose their reference points and swim with the current and/or are displaced downstream.

## VI. References

1. ALI, M. A. The ocular structure, retinomotor and photo-behavioral responses of juvenile Pacific salmon. Ph.D. Thesis, University of British Columbia, Vancouver, B.C. 1958.
2. AREY, L. B. The occurrence and the significance of photomechanical changes in the vertebrate retina—an historical survey. *J. Comp. Neurol.* **25**, 535-554 (1915).
3. AREY, L. B. Movements in the visual cells and retinal pigment of the lower vertebrates. *J. Comp. Neurol.* **26**, 121-201 (1916).
4. AREY, L. B. Visual cells and retinal pigment. In *Special cytology*, Vol. 2, Sect. 25, 887-926. Edited by E. V. Cowdry. Paul B. Hoeber Inc., New York. 1928.
5. AREY, L. B. and MUNDT, G. H. A persistent diurnal rhythm in visual cones. *Anat. Record*, **79** (Suppl.), 5 (Abstr.) (1941).
6. BRETT, J. R. and ALI, M. A. Some observations on the structure and photomechanical responses of the Pacific salmon retina. *J. Fisheries Research Board Can.* **15**, 815-829 (1958).
7. BRÜNNER, G. Über die Sehschärfe der Elritze (*Phoxinus laevis*) bei verschiedenen Helligkeiten. *Z. vergleich Physiol.* **21**, 296-316 (1934).
8. BUCKLAND, F. T. Fish hatching. Tinsley Brothers, London. 1863.
9. BUCKMANN, A., HARDER, W. S., and HAMPFEL, G. Unsere Beobachtungen am Hering (*Clupea harengus* L.). *Kurze Mitt. a.d. Fischereibiologie abt. d. Max-Planck-Instituts, Wilhelmshaven*, **3**, 22-42 (1953).
10. CLEMENS, W. A. On the migration of Pacific salmon (*Oncorhynchus*). *Trans. Roy. Soc. Can. Ser. 3*, **45** (Sect. V), 9-17 (1951).
11. CLEMENS, W. A. On some fundamental problems in the biology of Pacific salmon. *Trans. Roy. Soc. Can., Ser. 3*, **47** (Sect. V), 1-13 (1953).
12. CROZIER, W. J., WOLF, E., and ZERRAHN-WOLF, G. On critical frequency and critical illumination for reaction to flickering light. *J. Gen. Physiol.* **20**, 211-228 (1936).
13. DANNEVIG, A. Flodevigens Utklekningsansalt 1882-1932. IV Torsk, Utklekning og undersøkelse. *Årsberet. Vedkom. Norg. Fiskerier*, **4**, 17-30 (1932).
14. DETWILER, S. R. Vertebrate photoreceptors. The Macmillan Co., New York. 1943.
15. EIGENMANN, C. H. and SCHAFER, C. D. The mosaic of single and twin cones in the retina of fishes. *Am. Naturalist*, **34**, 109-118 (1900).
16. FRANZ, V. Einige Versuche zur biologie der fischlarven. *Intern. Revues. Hydrobiol. Hydrog.* **2**, 557-579 (1909).
17. FRANZ, V. *Sehorgan. Oppel's Lehrbuch der vergleichende mikroskopische Anatomie der Wirbeltiere.* G. Fischer, Jena. 1913.
18. GARTEN, S. Die Veränderungen der Netzhaut durch Licht. Von Graefe-Saemisch Handbuch der Augen heilkunde. Vol. 3, Chap. 12. 2nd ed. 1907.
19. GRAY, J. The growth of fish. II. The growth rate of the embryo of *Salmo fario*. *J. exptl. Biol.* **6**, 110-124 (1928).
20. HARRIS, J. E. and WOLFE, U. K. A laboratory study of vertical migration. *Proc. Roy. Soc. London, Ser. B*, **144**, 329-354 (1955).
21. HEIN, W. Zur Biologie der Forellenbrut I. Versuche über das Nahrungsbedürfnis der Bachforellenbrut im Brutrog und im Künstlichen Brutbett. *Allgem. Fisch. Etg.* **31**, 239-243 (1906).

22. HOAR, W. S. The behavior of chum, pink and coho salmon in relation to their seaward migration. J. Fisheries Research Board Can. **8**, 241-263 (1951).
23. HOAR, W. S. Control and timing of fish migration. Biol. Revs. **28**, 437-452 (1953).
24. HOAR, W. S. The behavior of migrating pink and chum salmon fry. J. Fisheries Research Board Can. **13**, 309-325 (1956).
25. HOAR, W. S. The evolution of migratory behavior among juvenile salmon of the genus *Oncorhynchus*. J. Fisheries Research Board Can. **15**, 391-428 (1958).
26. HOAR, W. S., KEENLEYSIDE, M. H. A., and GOODALL, R. G. Reactions of juvenile Pacific salmon to light. J. Fisheries Research Board Can. **14**, 815-830 (1957).
27. JONES, F. R. H. The behavior of minnows in relation to light intensity. J. Exptl. Biol. **33**, 271-281 (1956).
28. KAMPA, E. Effects of vitamin A supplementation and deprivation on visual sensitivity, rhodopsin concentration, and retinal histology of a marine teleost, *Gillichthys mirabilis*. Bull. Scripps Inst. Oceanogr. Univ. Calif. **6**, 199-224 (1953).
29. KEENLEYSIDE, M. H. A. Some aspects of the schooling behavior of fish. Behaviour, **8**, 183-248 (1955).
30. KOBAYASHI, H. Notes on retino-motor phenomena in some fishes under various light conditions. J. Shimonoseki Coll. Fish. **7**, 169-177 (1957).
31. McDONALD, J. G. The seasonal and diurnal movements of some Pacific salmon fry with particular reference to the sockeye (*Oncorhynchus nerka*). M. A. Thesis, University of British Columbia, Vancouver, B.C. 1956.
32. McEWAN, M. R. A comparison of the retina of the mormyrids with that of various other teleosts. Acta Zool. **19**, 427-465 (1938).
33. NEAVE, F. Notes on the sea-ward migration of the pink and chum salmon fry. J. Fisheries Research Board Can. **12**, 369-374 (1955).
34. PARKER, G. H. The movements of the retinal pigment. Ergeb. Biol. **9**, 239-291 (1932).
35. PICKFORD, G. E. and ATZ, J. W. The physiology of the pituitary gland of fishes. New York Zoological Society, New York. 1957.
36. POLYAK, S. L. The retina. The University of Chicago Press, Chicago. 1941.
37. POLYAK, S. L. The vertebrate visual system. The University of Chicago Press, Chicago. 1957.
38. ROCHON-DUVIGNEAUD, A. Les yeux et la vision des vertébrés. Masson et Cie, Paris. 1943.
39. SMITH, E. V. Effect of light on the development of young salmon. Publs. Puget Sound Biol. Sta. **1**, 89-107 (1916).
40. SOLEIM, P. A. Årsaker til rike og fattige åranger av sild Fisk. Skrifter Havrindersk. **7**, 22-24 (1942).
41. STUART, T. A. Spawning migration, reproduction and young stages of Loch trout (*Salmo trutta* L.). Sci. Invest. Fish. Scotland, **5**, 1-39 (1953).
42. STUDNITZ, G. VON. Beiträge zur Adaptation der Teleostear. (Studien zur vergleichenden Physiologie der Iris). II. Z. vergleich. Physiol. **18**, 307-338 (1933).
43. STUDNITZ, G. VON. Physiologie des Sehens (Retinale Primär-prozesse). Akademische Verlagsgesellschaft, Leipzig. 2nd ed. 1952.
44. TAVLOGA, W. N. Development of the gobiid fish, *Bathygobius soporator*. J. Morphol. **87**, 467-492 (1950).
45. VERRIER, M. L. Les variations morphologiques de la rétine et leurs conséquences physiologiques. A propos de la rétine d'une musaraigne (*Crocodyra mimula* Miller). Ann. sci. nat. Paris, **18**, 205-216 (1935).
46. WALLS, G. L. The vertebrate eye and its adaptive radiation. Cranbrook Institute of Science, Bull. **19**, Bloomfield Hills, Michigan. 1942.
47. WELSH, J. H. and OSBORN, C. M. Diurnal changes in the retina of the catfish, *Ameiurus nebulosus*. J. comp. Neurol. **66**, 349-359 (1937).
48. WHITE, G. M. The behavior of brook trout embryos from the time of hatching to the absorption of the yolk sac. J. Animal Behaviour, **5**, 44-60 (1915).
49. WIGGER, H. Versuche zur Kausalanalyse der retinomotorischen Erscheinungen. Pflüger's Arch. ges. Physiol. **239**, 215-246 (1937).
50. WIGGER, H. Diskontinuität und Tagesrhythmus in der Dunkelwanderung Retinaler Elemente. Z. vergleich. Physiol. **28**, 421-427 (1941).
51. WOLFE, E. and ZERRAHN-WOLF, G. Threshold intensity of illumination and flicker frequency for the eye of the sunfish. J. Gen. Physiol. **19**, 495-502 (1935).
52. WOODHEAD, P. M. J. Reactions of salmonid larvae to light. J. Exptl. Biol. **34**, 402-417 (1957).
53. YAMANOUCHI, T. The visual acuity of the coral fish, *Microcanthus strigatus* (Cuvier and Valenciennes). Publs. Seto. marine Biol. Lab. **5**, 133-156 (1956).

# THE OSMOREGULATORY ROLE OF THE THYROID GLAND IN THE STARRY FLOUNDER, *PLATICHTHYS STELLATUS*<sup>1</sup>

CLEVELAND P. HICKMAN, JR.<sup>2</sup>

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<sup>1</sup>Manuscript received February 23, 1959.

Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C. Financial support for this research was received from the National Research Council of Canada through grants-in-aid of research to W. S. Hoar and a studentship to C.P. Hickman, Jr.

<sup>2</sup>Present address: Department of Zoology, University of Alberta, Edmonton, Alta.

### Abstract

Energy demands for osmotic regulation and the possible osmoregulatory role of the thyroid gland were investigated in the euryhaline starry flounder, *Platichthys stellatus*. Using a melting-point technique, it was established that flounder could regulate body fluid concentration independently of widely divergent environmental salinities. Small flounder experienced more rapid disturbances of body fluid concentration than large flounder after abrupt salinity alterations.

The standard metabolic rate of flounder adapted to fresh water was consistently and significantly less than that of marine flounder. In supernormal salinities standard metabolic rate was significantly greater than in normal sea water. These findings agree with the theory that energy demands for active electrolyte transport are greater in sea water than fresh water.

Thyroid activity was studied in flounder adapted to fresh water and salt water. Percentage uptake of radioiodine by the thyroid was shown to be an insensitive and inaccurate criterion for evaluating thyroid activity in different salinities because removal rates of radioiodine from the body and blood differed between fresh water and marine flounder. Using thyroid clearance of radioiodine from the blood as a measure of activity, salt-water flounder were shown to have much greater thyroid clearance rates and, hence, more active thyroid glands than flounder adapted to fresh water. The greater activity of the thyroid of marine flounder correlates with greater oxygen demands in sea water and suggests a direct or adjunctive osmoregulatory role of the thyroid gland of fish.

### Introduction

The role of the endocrines in salt and water metabolism of fishes is reviewed by Pickford and Atz (93). No major osmoregulatory function has been demonstrated conclusively for the endocrine glands, although the evidence is insufficient to dismiss such a function for any of them. Often implicated in an osmoregulatory role is the enigmatic thyroid gland. However, its precise action in this regard has remained obscure. Even more puzzling has been the inability of numerous workers to demonstrate in teleosts the fundamental calorogenic action of the thyroid of higher vertebrates.

The present investigation is a study of the osmoregulatory metabolism of a euryhaline teleost, the starry flounder (*Platichthys stellatus*), subjected to varied environmental salinities, with particular reference to the role of the thyroid gland. This investigation is based on the assumption that if energy demands for osmotic regulation are mitigated or intensified by changing the ambient salinity, such changes should be reflected in a concomitant increase or decrease in both total metabolic rate and thyroid activity. Experimental evidence will be presented to show that energy demands of flounder are consistently and significantly greater in sea water than fresh water, and that the activity of the thyroid gland as evaluated by thyroïdal clearance rates of radioiodine from the blood is greater in sea-water-adapted than fresh-water-adapted flounder.

The starry flounder was chosen as the experimental animal for this study because, in addition to possessing the attribute of euryhalinity, it is a hardy, rugged fish that lends itself exceptionally well to experimental treatment. It is relatively unexcitable, and could be readily collected from its marine environment during most of the year. Occasionally, other species of flatfish were collected, and isolated comparative experiments were performed when possible.

This work is presented in three sections: (1) establishment of the osmotic capacity in terms of the ability to regulate body fluid concentration in widely divergent salinities, (2) the relative energy demands for osmotic regulation, and (3) the effect of environmental salinity on thyroid activity.

### Collection and Care of Fish

Experimental work was carried out during 2 years—from the summer of 1956 to summer 1958. The laboratory facilities of the Vancouver Public Aquarium were utilized for most of the work. The salinity of the sea water in the aquarium varied seasonally. During the winter it remained high, 25–27‰, but in the early summer it dropped to as low as 16‰. The salinity rarely rose above 20‰ until fall.

Initially, flounder were collected at frequent intervals by means of a beach seine at Locarno Beach, Vancouver. Subsequently, all flounder were caught by a small otter trawl on the North Bank of the Fraser River, Steveston, British Columbia. Both of these areas are characterized by low salinities in the summertime, the Locarno Beach area as low as 10‰ and the North Bank of the Fraser as low as 6‰. Flounder were held in tanks containing about 130 liters of running sea water at a temperature within 1.0° C of the environmental temperature at the collection locale. After 2 or 3 days, the flounder were transferred to similar tanks of water adjusted to the desired experimental salinity. To minimize the effect of thermal acclimation, the temperature was maintained within  $\pm 1.0^\circ$  C of the environmental temperature. Water was changed if any sign of fouling appeared. The tanks were partially covered, but daylight was not excluded. The fish appeared to thrive under these conditions and virtually no mortality occurred between the time of capture and the end of the experiments (2 weeks or less).

Since the metabolism of foodstuffs forms a large portion of the total calorific output of animals, it is usually found advantageous to fast experimental animals before measuring standard<sup>3</sup> metabolism. Accordingly, none of the flatfish were fed in the laboratory. There is no evidence that the drop in metabolic rate, caused by the cessation of food assimilation and protein storage for growth, would in any way interfere with the fish's capacity to perform osmotic work. In the present experiments, although starved flounder lived for 1½ months in the summer and about 3 months in the winter before appreciable mortality began, all experimental work was begun at least within 10 days (usually within 4 days) and terminated within 15 days after collection of the fish.

<sup>3</sup>Basal metabolism, a term in common usage by medical physiologists and clinicians, refers to the metabolic rate of a resting (but not sleeping) and fasted animal, removed from discomfort and distracting influences. Because the metabolism of vertebrates can be lowered from the "basal" rate by drugs or sleep, Krogh (66) suggested the term standard metabolism for the energy requirements under normal resting conditions. Standard metabolism would seem to be a more accurate term and has been adopted by most comparative physiologists studying energy metabolism in animals.

### Osmotic Response to Changes in Environmental Salinity

In fishes, the principal organs for osmotic exchange are the kidneys and the gills. Fresh-water fish depend primarily for the prevention of overhydration on the excretion of a copious and dilute urine, whereas the kidney is more of a liability than an asset to the marine teleost. In the gills of the marine teleost are cells capable of concentrating and excreting ions to the exterior. In spite of the opposite directions of active salt and water exchange in fresh- and salt-water fishes (overhydration vs. dehydration), both regulate the osmolar concentration of their body fluids at comparable levels: fresh water fishes at  $\Delta$  0.5 to 0.7° C and marine fishes at about  $\Delta$  0.7 to 0.9° C (15). Reported values for body fluid osmolarity of fishes vary greatly between species and often within a species. Interspecific variations are due to several contributing factors such as error in various methods of measurement, and abnormal changes in plasma osmolarity due to "laboratory diuresis" (31). In addition, body fluid osmolarity is never maintained absolutely constant even under relatively stable environmental conditions. A certain amount of lability is to be expected, particularly if the osmotic gradient changes.

Evaluation of euryhalinity usually is based on the ability of fish to survive a wide range of salinities. Such a criterion is crude, for it cannot reveal the extent of disturbance of body fluid composition and concentration, hence the degree of perfection of homeostatic mechanisms. Presented in this section is a precise method for describing the ability of an aquatic animal to regulate under a wide range of concentration gradients by a melting-point technique.

#### DETERMINATION OF BODY FLUID CONCENTRATION

##### *Melting-point Determination*

Total osmotic pressure of serum and urine was determined by a melting-point method similar to that used by Gross (42). This method involves comparison of the time of melting of frozen solutions of unknown melting point with solutions of known melting point, when allowed to warm very slowly and linearly in a cold brine bath. Because only enough solution (.01-.001 ml) is needed to fill a small portion of a capillary tube, the method is particularly desirable where small quantities of fluid are available. For this study, this method offered the following advantages: (a) it is applicable for osmoconcentration measurements of body fluids of small fish (less than 10 g) as well as large; (b) urinary catheterization is unnecessary since only a drop of urine is needed—an amount easily expressed by gentle pressure over the urinary bladder; and (c) many of the problems encountered in freezing-point determinations are eliminated, e.g. coagulation and supercooling.

The brine bath consisted of a 2-liter capacity plastic box insulated with cork and rock wool. Windows were provided on the top and bottom of the chamber for illumination and observation of the tubes. Standard sodium

chloride solutions with melting points of about  $-2^{\circ}$ ,  $-1^{\circ}$ ,  $-0.5^{\circ}$ , and  $0^{\circ}$  C were prepared and the exact osmotic concentrations of each determined to an accuracy of  $.005^{\circ}$  C with a differential thermometer. In an experimental run, capillary tubes containing approximately equal quantities of the unknowns were quick-frozen on dry ice and placed side by side on a rack in the brine bath at an initial temperature of  $-9.0^{\circ}$  C. Beside these were placed capillary tubes containing equal quantities of the four standard solutions, similarly frozen on dry ice. The brine bath was then covered and stirred gently during the period of warming. Warming was rapid initially, but soon slowed. During the critical period ( $-2.0$  to  $0^{\circ}$  C) the temperature rose about  $1^{\circ}$  C every 35 minutes in a nearly linear fashion. The tubes were watched with a microscope and the time of melting of both standard and unknowns recorded by marking a kymograph drum with signal magnets arranged for the purpose. Then, by plotting and fitting a line through the points, the melting-points of the unknowns could be interpreted relative to those of the standards. The method used here had an accuracy to within  $.01^{\circ}$  C, a little less than the accuracy possible to achieve by careful freezing-point determinations ( $.005^{\circ}$  C).

#### *Experimental Procedure*

It has been shown frequently that marine fish held in overcrowded conditions in the laboratory or subjected to handling develop a "laboratory" or "osmotic diuresis" that may persist for a long period of time (31, 32). Evidence indicates that a spontaneous increase in urine flow occurs immediately after capture as the result of trauma imposed during capturing, holding, and handling. In this investigation, single urine samples were collected from each fish, thus eliminating two important contributing factors to severe laboratory diuresis: bladder catheterization and the continual handling necessary for serial sampling of individual fish.

After capture, fish were held for 3 days in flowing sea water at a temperature of  $14-15^{\circ}$  C. At the beginning of the experiment, a group of flounder was transferred from the holding tank to the desired salinity. Controls (flounder maintained in normal,  $25\text{‰}$ , sea water) also were transferred to a similar aquarium so that effects due to handling would be the same in both control and experimental fish. At selected intervals following transfer, fish were removed, rinsed in fresh water, and blotted dry. A urine sample was collected by touching the tip of a capillary tube to the urinary papillae and pressing gently over the urinary bladder. The drop of urine was tapped to the center of the tube, the tube sealed at both ends with a heavy inert grease (Nevastane, Heavy X, Keystone Co.), and immediately placed on dry ice. Blood was collected by puncturing the dorsal aorta above and slightly posterior to the center of the coelom with a narrow, sharp-pointed scalpel blade. Because of the lateral compression of flatfish, the dorsal aorta lies relatively near the surface and may be easily punctured from the side of the animal without entering either the coelom or spinal cord. By inverting the animal, blood was allowed to flow onto a clean glass slide and left undisturbed until



it had clotted (20–50 seconds). Serum was drawn into the capillary tube and the tube sealed and frozen. Melting points were determined either immediately or after brief storage in a brine solution at  $-10^{\circ}\text{C}$ .

## RESULTS

### *Starry Flounder in Normal Sea Water*

At the beginning of the experiment the osmoconcentration of the serum of flounder in 25‰ sea water averages  $\Delta 0.69^{\circ}\text{C}$  (Fig. 1, Table I). At 76 hours the average serum osmolarity has increased to  $\Delta 0.702^{\circ}\text{C}$  and to  $\Delta 0.706^{\circ}\text{C}$  at 14 days. The urine melting-point was invariably lower than the serum melting-point of the same fish, as has been repeatedly demonstrated for other marine teleosts (15).

It is notable that with the large increase in variability of serum and urine concentration at 14 days, urine always remains hypotonic to the serum when values are compared on an individual basis. In comparing relative degrees of hypotonicity of urine to serum, the urine:serum melting-point ratio is useful. These values have been calculated and are included in Table I. The average U/S ratios of the initial and 76-hour samples are essentially the same (.834 and .835), but the average 14-day U/S ratio has increased to .91. Forster and Berglund (31, 32) have shown that the onset of laboratory diuresis is accompanied by a shift in total electrolyte composition of plasma and urine and an increase in urine flow and loss of chloride. These factors contribute to a marked increase in tonicity of both urine and extracellular fluid. They noted a progressive rise in the urine:plasma ratio during laboratory diuresis and eventually blood and urine become isotonic. Inasmuch as the U/S ratio of flounder in this study increased not at all between 0 and 76 hours and only slightly at 14 days, it is unlikely a laboratory diuresis developed during the experiment. It is conceivable, nevertheless, that a change had occurred in the U/S ratio in the 3-day interval between capture and initial sampling.

### *Starry Flounder in Hypotonic Media: Regulation against Overhydration and Salt Depletion*

Figure 1 shows that abrupt transfer to hypotonic media results in an immediate dilution of the blood. The average serum osmolarities of flounder in both fresh water and hypotonic sea water are lower at 5 than at 12 hours, suggesting that the initial influx of water into the extracellular space exceeds the capacity of the kidney to excrete the excess fluid. A brief recovery occurred at 12 hours and was followed by a slower drop in body fluid concentration until a balance was struck between water influx and output. This occurred within 24 hours for flounder in 5.45‰ sea water but the concentration continued to drop in fresh-water flounder until equilibrium was reached—sometime between the first and third days following transfer.

The concentration of the urine decreased markedly during the first day (Fig. 1). Surprisingly, the drop in urine concentration of flounder in dilute sea water is initially greater than that of fresh-water flounder. As would

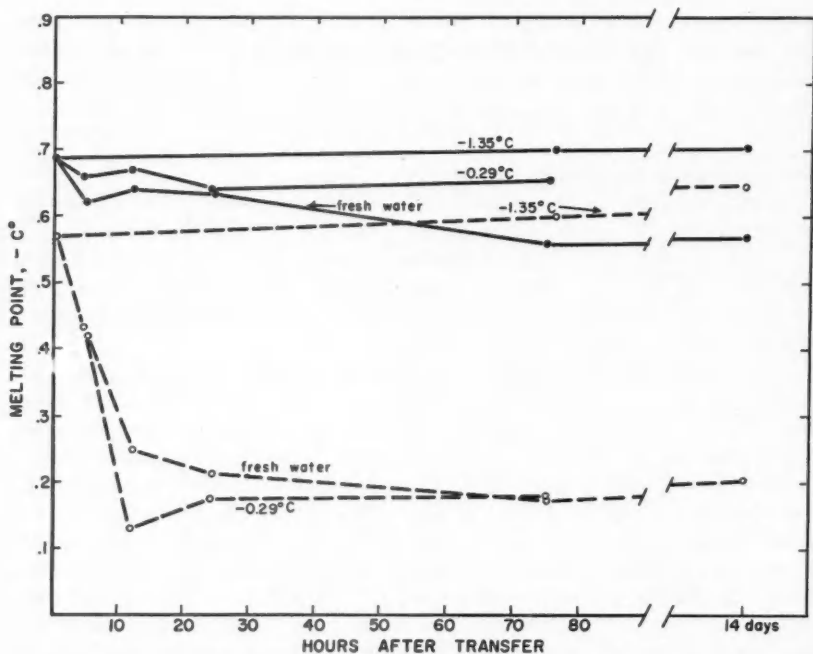


FIG. 1. Average melting points of serum (solid lines) and urine (broken line) of *Platichthys stellatus* transferred abruptly from sea water of 25‰ ( $\Delta 1.35^\circ\text{C}$ ) to dilute sea water of 5.45‰ ( $\Delta 0.29^\circ\text{C}$ ) and fresh water. Individual values summarized in Table I.

be expected, urine concentration reached equilibrium at about the same time as did the blood concentration, i.e., 24 hours for flounder in dilute sea water and about 3 days for fresh-water flounder. The slight increase in osmolarity of the serum and urine of the flounder in fresh water between the 3rd and 14th days is probably the result of a net retention of solutes with inanition as demonstrated with goldfish by Meyer, Westfall, and Platner (80) and Jørgensen and Rosenkilde (58).

Starry flounder tolerate abrupt transfer from sea to fresh water with absolutely no outward appearance of distress or asthenia. In fresh water they remain alert and vigorous under the added imposition of starvation for many weeks. Body volume increased somewhat after transfer to fresh water, but no excessive hydration was apparent until after several weeks of inanition at  $15^\circ\text{C}$ , when mortality began. The immediate and direct cause of death appeared almost invariably to be the result of a breakdown in osmotic regulation. A noticeable edema occurred and was accompanied by a stiffening of the body and a marked decrease in swimming activity when disturbed. Moribund fish were occasionally so stiff that they could hardly be forcefully bent when picked up.

Also characteristic of osmotic failure were cardiovascular disturbances. Collection of blood samples became increasingly difficult, suggesting a marked decrease in blood volume and/or cardiac output, producing a near circulatory stasis. Such critical changes in circulation occurred during the terminal stages of osmotic failure, but some small decrease in the effective blood flow was occasionally witnessed in healthy fresh-water flounder and appeared to be a normal consequence of the relative over-hydration of these animals.

*Starry Flounder in Supernormal Hypertonic Media: Regulation against Dehydration and Salt Excess*

The blood and urine osmolarities quickly rise when flounder are abruptly transferred from the control salinity of 25‰ ( $\Delta 1.35^\circ \text{C}$ ) to concentrated sea water of 46‰ ( $\Delta 2.49^\circ \text{C}$ ) (Table I). The average serum melting point drops from  $\Delta 0.69$  to  $\Delta 0.76^\circ \text{C}$  during the first  $4\frac{1}{2}$  hours, a decrease of about 10% (10% increase in total osmotic pressure). Between  $4\frac{1}{2}$  and 12 hours the concentration changes very little, but is then followed by a gradual continuous rise to 74 hours, when the experiment was terminated. Whether the brief pause between  $4\frac{1}{2}$  and 12 hours has special significance is not known, though a similar "recover" period was noted in the progressive decrease in blood concentration of flounder transferred to fresh water.

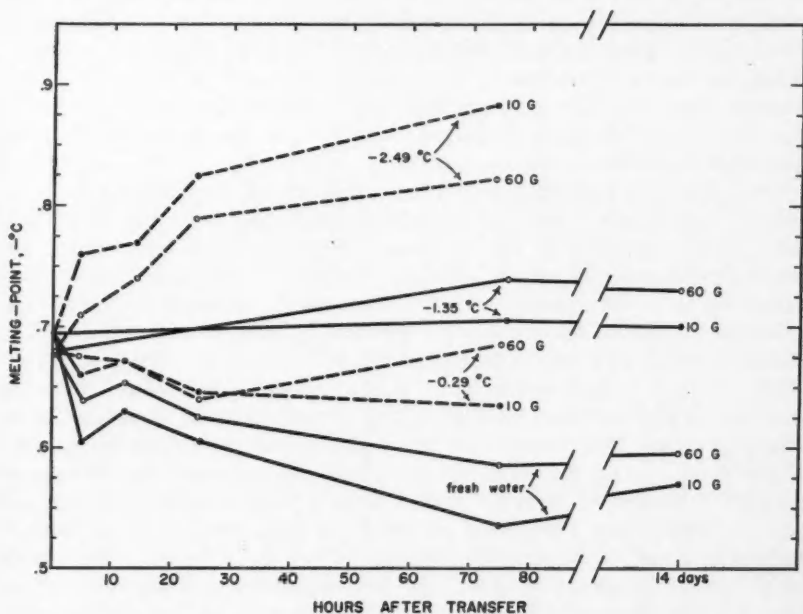


FIG. 2. The effect of body size on the rapidity of change of serum melting point of *Platichthys stellatus* transferred abruptly from sea water of 25‰ to concentrated sea water of 46‰, to dilute sea water of 5.45‰, and to fresh water.

*Effect of Body Size on Alterations in Body Fluid Osmolarity after Abrupt Changes in Environmental Salinity*

To assess the effect of size on the rapidity of concentration changes affected by abrupt salinity alterations, the individual melting points of serum were plotted on a linear axis against body weight on a logarithmic axis for each sampling period (Fig. 2). From each eye-fitted line through the values, two points were graphically derived representing the serum melting points of a 10-g and a 60-g starry flounder and plotted against time after transfer. It is evident from Fig. 2 that the osmotic disturbance following a salinity change is considerably greater in smaller flounder. The body fluid concentration of smaller flounder is shifted more rapidly and to greater degree than large flounder in the direction of the osmotic concentration of the external media, i.e., serum osmolarities are greater in concentrated sea water and less in fresh water in smaller individuals.

### Oxygen Consumption

Osmotic independence of living organisms appears to have originated a number of times during animal evolution and radiated along several lines resulting in the appearance of rather diverse regulatory mechanisms to solve osmotic problems encountered in nature. Because of such diversity, it is not surprising that investigations of energy output associated with osmotic work in different groups of aquatic animals have resulted in a seemingly irreconcilable array of experimental findings. Table II presents a summary of the literature on the subject. It is obviously not possible to fit all of these findings, some of which seem contradictory, into one picture. In addition, the variety of techniques employed contribute to the inadequacy of the results for comparison with one another.

Deviations in metabolic rate as affected by salinity have not always been accepted as indicative of altered energy demands for osmotic work. Schlieper (102, 103), in particular, has proposed alternate theories to account for respiratory changes in marine animals, primarily invertebrates, exposed to variations in environmental salinity. In general, Schlieper's theories are attempts to explain the frequently observed increase in metabolic rate of marine invertebrates moved into brackish or fresh water. His first theory (102), which he himself subsequently refuted, assumed that oxygen demands were less in high salinities because carbon dioxide could be eliminated more efficiently in salt than fresh water, thus reducing the carbon dioxide content of the blood. In the second theory (103) Schlieper proposed that the oxygen content of tissues was directly related to their water content. In low salt concentrations water would flood and swell the cells, thereby increasing their surface area and facilitating the exchange of respiratory gases. Arguments have been raised against the theory (69) but it does offer a convenient explanation for the oft-observed increase in respiration of marine poikilosmotic animals introduced into low salinities.

It is paramount in any study of the energetics of osmotic regulation to appreciate fully the osmotic capabilities of the species. Measurements of

TABLE II  
The influence of salinity on oxygen consumption of teleost fish as reported in the literature

| Species  | Habitat                          | Regulatory capacity                | Salinity effect   | Author                    |
|--|----------------------------------|------------------------------------|---|---------------------------|
| <i>Salmo irideus</i> and <i>Salmo solar</i> (eggs and alevins) | Fresh water                      | Hyperosmotic regulator             | Salinity change has no effect. During growth O <sub>2</sub> consumption increases more rapidly in fresh water   | Busnel <i>et al.</i> (18) |
| <i>Tinca vulgaris</i>  | Fresh water                      | Stenohaline hyperosmotic regulator | Transfer to salinities of 10 to 15 ‰ results in gradual fall in O <sub>2</sub> consumption to death, more rapid in higher concentrations. Death apparently by asphyxiation  | Cordier and Maurice (26)  |
| <i>Tinca tinca</i>   | Fresh water                      | Stenohaline hyperosmotic regulator | Increased salinity caused fall in O <sub>2</sub> consumption followed by death  | Raffy (95, 96)            |
| <i>Carassius carassius</i>                                     | Fresh water                      | Stenohaline hyperosmotic regulator | In salinities up to isotonicity, O <sub>2</sub> consumption increased. In hypertonic media, O <sub>2</sub> consumption decreased to 60% of normal   | Veselov (118)             |
| <i>Anguilla vulgaris</i> (juvenile)                            | Migrating sea to fresh water     | Euryhaline                         | O <sub>2</sub> consumption less in fresh water  | Raffy and Fontaine (98)   |
| <i>Anguilla vulgaris</i> (adult)                               | Migrating fresh to sea water     | Euryhaline                         | O <sub>2</sub> consumption less in fresh water  | Raffy (96)                |
| <i>Fundulus porciptinus</i>                                    | Marine                           | Euryhaline                         | Abrupt transfer to fresh water causes decrease in O <sub>2</sub> consumption. Returns to normal after fresh-water acclimation   | Keys (64)                 |
| <i>Gasterosteus</i>  | Fresh water                      | Euryhaline                         | O <sub>2</sub> consumption 20-30% higher in fresh water than isotonic sea water. No further decrease with increase in salinity  | Graetz (41)               |
| <i>Coregonus sardinella</i>                                    | Fresh water and marine migratory | Euryhaline                         | Marine (migratory) forms have higher metabolic rates than fresh-water forms   | Wohlschlag (121)          |
| <i>Scorpaena porcus</i>  | Marine                           | Stenohaline hyperosmotic regulator | Transfer to fresh water causes immediate drop in respiration to half normal sea-water rate, then followed decline to death  | Cordier and Leblanc (25)  |
| <i>Scyllium canis</i>  | Marine                           | Stenohaline hyperosmotic regulator | Transfer to dilute sea water or fresh water causes O <sub>2</sub> consumption drop followed by death  | Raffy (95, 96)            |
| <i>Sargus rondelleti</i>                                       | Marine                           | Stenohaline hyperosmotic regulator | Transferred to dilute sea water, O <sub>2</sub> consumption at first rises, then drops suddenly followed by death   | Raffy (96)                |
| <i>Pleuronectes platessa</i> (juvenile)                        | Marine                           | Hypo-osmotic regulator             | Transfer to dilute sea water and fresh water had no significant effect on oxygen consumption  | Raffy (97)                |
| <i>Pleuronectes platessa</i> (adult)                           | Marine                           | Hypo-osmotic regulator             | Low salinities cause increased O <sub>2</sub> consumption   | Henschel (47)             |
| <i>Hippocampus</i>   | Marine                           | Stenohaline hypo-osmotic regulator | Transfer to dilute sea water causes transitory increase in respiration. Large dilution causes continual fall. In concentrated sea water, O <sub>2</sub> consumption rises when fish returns to normal. If too saline, respiration falls continually | Leiner (72)               |

oxygen consumption of a fish subjected to a salinity beyond that which it can indefinitely tolerate have doubtful significance, because the observed changes may be of a pathological nature. Even in euryhaline forms, a change in the energy requirements concomitant with salinity changes must be interpreted with caution. Transitory increases in oxygen consumption usually are measured when a fish is introduced into a new salinity and may be the result of the stress imposed. It is important, therefore, that the animal be adapted to the experimental salinities before definitive determinations of oxygen consumption are made. In the published literature on the effect of salinity on the oxygen consumption of fishes, it would appear that inadequate attention has been paid to salinity adaptation and the osmotic capacity of the species.

The indirect determination of metabolic rate in fishes as measured by the consumption of dissolved oxygen must be carried out with certain precautions. Fortunately the problems of such measurement have recently become appreciated and the unrecognized pitfalls encountered by the earlier workers can now be largely avoided. The most important considerations discussed in a recent review by Fry (35) are:

- |   |                              |
|---|------------------------------|
| (1) body size,                                    | (5) diurnal variations,      |
| (2) experimental temperature and thermal history, | (6) activity,                |
| (3) oxygen and carbon dioxide tensions,           | (7) nutritional state,       |
| (4) seasonal influences,                          | (8) sex and sexual maturity, |
|   | (9) environmental salinity.  |

In this investigation the attempt has been made to control, eliminate, or take into account all of these influences, leaving one variable, salinity, to be experimentally altered. Because the effect of size on the metabolic rate - thyroid activity interrelationship formed an important part of the investigation, a fairly large size range of flounder (about 4 to 300 g) was used. Among the important advantages of using a wide range of sizes are that the data lend themselves well to statistical treatment and yield a great deal more information regarding the influences of the experimental variable. In addition, important quantitative differences in the effect of salinity between small and large fish may come to light.

In this discussion, the terms metabolism, total metabolism, oxygen consumption, oxygen uptake, and respiration will refer to "oxygen consumption *per fish* per hour". The terms metabolic rate, respiratory rate, respiratory intensity, weight-specific oxygen consumption, and rate of oxygen consumption will mean "oxygen consumption *per gram body weight* per hour".

#### DETERMINATION OF STANDARD METABOLIC RATE

##### *Apparatus*

Standard metabolism was measured in an apparatus utilizing the constant flow principle described by Keys (63). Because a variety of experimental salinities was needed, the apparatus used here was modified so that the water of the desired salinity could be continuously recirculated. The respiratory

chambers, containing individual fish, were submerged side by side in an insulated respirometer bath, 264 cm long by 25 cm wide. Water from an elevated 120-liter barrel entered the bath at one end through a constant-level bottle and left by an overflow at the other. Overflow water and outflowing water from the respirometers was collected in a 100-liter tank below and pumped automatically to the reservoir above.

Water was cooled in the collecting tank with a series of cooling tubes and held thermostatically at the desired temperature. During an entire experimental run (about 24 hours) the temperature varied less than  $0.4^{\circ}\text{C}$  and less than  $0.1^{\circ}\text{C}$  from one end of the respirometer bath to the other at any one time. The circulating water was vigorously aerated in the collecting tank and repeated analyses showed that the oxygen tension in the bath was always at air saturation.

Because flatfish are laterally compressed they present a special problem in the selection of a respiration chamber. Clear Lucite refrigerator boxes manufactured by Tri-State Plastics, Louisville, Kentucky, proved suitable. Four sizes were selected which would accommodate a size range of flounder up to 300 g and lemon sole to 200 g. The dimensions and capacities of the chambers were:  $26.3 \times 19 \times 10.2$  mm (5.1 liters),  $18.6 \times 13 \times 10.2$  mm (2.47 liters),  $16.7 \times 11.8 \times 6.7$  mm (1.32 liters),  $11.8 \times 8.2 \times 6.6$  mm (0.64 liters). The boxes were drilled at one end and a two-hole rubber stopper was fitted with an inflow tube passing the length of the box to the opposite end. The outflow tube was connected to a length of tygon tubing which siphoned the effluent over the edge of the trough and into a sample bottle (30-ml glass-stoppered Erlenmeyer flask). Rate of water flow through each respirometer was controlled by raising or lowering the sample bottle with a number of thin plywood shims. This method was found superior to regulating the flow with a clamp, which tended to trap air bubbles or excreta, resulting in tube blockage. Just prior to collecting water samples for oxygen analysis, flow rates were measured by collecting the overflows from the sample bottles in graduate cylinders. The error between duplicate flow measurements was usually less than 1% for the large respirometers with a fast flow and up to 3% for the small respirometers with a relatively slow flow.

The respirometer used corresponded roughly to the size of fish, with a volume of water in the chamber 15 to 100 times the volume of fish. In accord with Geyer and Mann (37), who found that a respiration chamber with a volume smaller than 10 times that of the fish caused overexcitement of *Perca fluviatilis*, it was noted that a chamber at least 10 times the volume of flounder was necessary to avoid heightened respiration. A layer of washed, screened sand was placed in the bottom of each respirometer. The flatfish usually buried themselves in the sand, leaving only the upper surface of the head and operculum exposed.

#### Chemical Analysis

Dissolved oxygen was determined by a semimicro modification of Winkler's iodometric technique, observing the precautions discussed by Ohle (84). A

20-ml aliquot of the sample was titrated, using a microburette and a dilute (.01 *N*) thiosulphate solution. The error in repeated titrations of one fixed sample was less than 1%.

#### *Experimental Procedure*

A number of workers (14, 63, 119) have demonstrated that the handling of fish necessary to place them in the experimental chamber creates a greatly heightened oxygen consumption which subsides only gradually. For this reason, it is necessary to maintain the fish several hours under controlled conditions before the first samples are drawn. Fish were introduced into the respirometers the evening before the day that measurements were made. They thus had a period of 18 to 22 hours in the chambers under constant experimental conditions (darkness, quiet, and constant temperature, salinity, and water flow).

Another influence which tends to supplement the physiological variation in metabolic rate already present is that of endogenous 24-hour cycles in respiratory intensity. Endogenous cycles have been found in fish respiration by a number of workers (35). Pilot experiments showed the presence of such a diurnal rhythm in starry flounder, with consumption high in the morning, decreasing measurably until about 1:00 p.m., and leveling off during the remainder of the afternoon until about 6 p.m., when it increased sharply. To minimize this influence and ensure basal conditions all samples were collected during the afternoon.

The oxygen consumption of 12 to 18 fish was determined during each experimental run. After the water in the apparatus was adjusted to the desired experimental salinity and temperature, the fish were transferred quickly from the holding tank to the respirometers. The respirometer tops were then sealed with a heavy, non-toxic grease (Nevastane, Heavy X, Keystone Co.). When all fish were in place, the trough was covered and flow rates adjusted so that under conditions of standard metabolism the concentration of dissolved oxygen in the water leaving the respirometers would be between 70 and 85% air saturation. At 1 and 3 p.m. of the following afternoon, flow rates were measured and samples of outflowing water from each respirometer were immediately collected, fixed, and analyzed for dissolved oxygen. A third sample was collected and analyzed if the first two differed in excess of about 5%. At these same times three samples were drawn for analysis of the water at both ends and in the center of the trough (inflowing water). After completion of the experiment, the fish were removed from respirometers, weighed (wet weight with bodies blotted), and returned to the holding tanks.

#### *Statistical Procedures*

Rate of oxygen consumption was plotted as a function of body weight (weight-specific) in a double logarithmic coordinate system. This method was selected over the other common mode of presentation, i.e., logarithm of total oxygen consumption against logarithm of body weight, because it graphically emphasizes the weight dependence of respiratory intensity.

When plotted on a double logarithm grid, weight-specific oxygen consumption of animals over an adequate size range depicts a straight or nearly straight line with a negative slope. Regression of total oxygen consumption against body weight is of the form:

$$O_2 = aW^b$$

or

$$\log O_2 = \log a + b \log W$$

where  $O_2$  is total oxygen consumption of the fish in mg  $O_2$ /g/hr,  $W$  is body weight in grams,  $a$  the intercept, and  $b$  the slope of the line. Since rate of oxygen consumption was preferred for presentation here, the equation was transformed to weight-specific by dividing by  $W$ . Thus:

$$O_2/W = aW^{(b-1)}$$

or

$$\log O_2 - \log W = \log a + b \log W - \log W.$$

The problem here was to test whether statistically significant differences existed between two regression lines, representing the metabolic rates of flatfish measured in two different salinities. Usually oxygen consumption of 12 to 24 fish was determined for each experimental salinity during a test series and often considerable variability existed around each calculated regression line. Data of this nature are readily adapted to statistical treatment by analysis of covariance. The procedure was to test by analysis of covariance the null hypothesis that no true differences existed in the effect of two salinities on oxygen consumption, i.e., that the two regression lines could be represented just as well by a single regression line. To eliminate negative log  $Y$  values in the statistical analysis, oxygen consumption rates were multiplied by 100 before converting to common 5-place logarithms. A standard method of covariance analysis outlined by Ostle (91) for a randomized design was followed.

## RESULTS<sup>4</sup>

### *Standard Metabolic Rates*

#### *Interspecific Comparison*

In Fig. 3 a graphic comparison is made of standard metabolic rates of flounder, sole, and sand dab in sea water of 25‰. Measurements were made at a temperature of 15°C and at a time when all species were in a similar nutritional state (3-4 days of fasting).

Weight-specific oxygen consumption of flounder and sole are essentially the same throughout the size range examined but greater than the metabolic rate of sand dab at an equivalent body size. In addition, there exists a more rapid depression of metabolic rate with increasing size of flounder and sole ( $b-1 = -.141$  and  $-.158$  respectively) than of sand dab ( $b-1 = -.095$ ). These values correspond to " $b$ " values of .859, .842, and .905

<sup>4</sup>The original data from which the representative figures in this section were derived are tabulated in a doctoral thesis by the author and deposited in the library of the University of British Columbia.

respectively. However, it is important to recognize that one is dealing with an interspecific comparison of fish that differ markedly with respect to ultimate size attained and size at sexual maturation. Included in Fig. 3 is an indication of the size of each species at first maturity. In both starry flounder and lemon sole, males usually reach maturity a year ahead of females (a general phenomena among fishes), roughly at 200–250 g body weight (61, 62, 89). Starry flounder grow very large, in excess of 9 kg and lemon sole to perhaps 3 kg. The sand dab, on the other hand, is a small species. Sexually mature females of 20 g were occasionally found, and the largest individuals of the species rarely exceed 50 g. Hence, with respect to the ultimate sizes of the three species, it is clear that altogether different periods of ontogeny are represented in the metabolism curves.

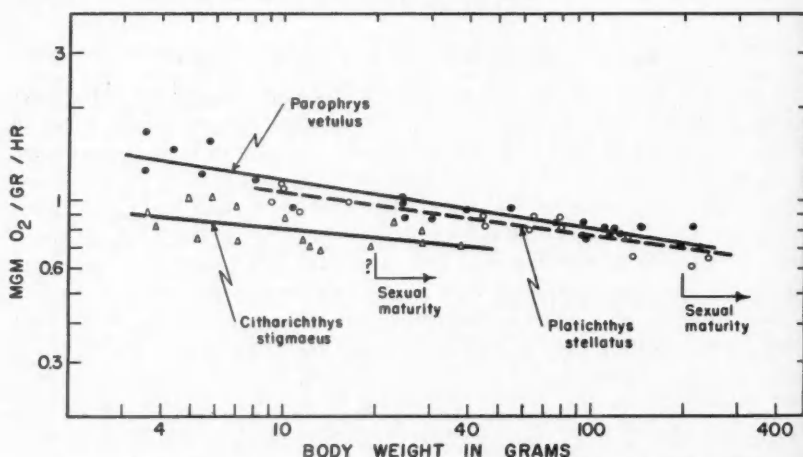


FIG. 3. Standard metabolic rate of *Platichthys stellatus* (open circles), *Parophrys vetulus* (closed circles), and *Citharichthys stigmaeus* (triangles) in sea water of 20–25°C. Experimental temperature 14–15°C. Lines are fitted by the method of least squares.

Figure 3 shows that the respiratory intensity of sand dab at maturation (about .075 mg O<sub>2</sub>/g/hr) is essentially the same as that of sole and starry flounder at maturation (about .070 mg O<sub>2</sub>/g/hr). Whether or not the metabolic rate of these species is actually more dependent on the rate of growth and physiological age than upon body size, per se, will have to await further experimentation.

#### Significance of the Slope of Regression of Metabolic Rate

The relationship of metabolism to body size as expressed by the formula

$$O_2 = a \text{ Weight}^b$$

indicates a weight or allometric dependence of oxygen consumption of animals. According to the surface concept, metabolism is a 2/3 power of body weight. Plotting oxygen consumption as a function of body weight on a double logarithmic grid, the slope of a surface proportional curve is 0.67 as opposed to a

slope of 1.0 for weight proportionality. Plotted as weight-specific metabolism the slopes of regression for weight and surface proportionalities are 0 and -0.33 respectively.

Slopes of weight-specific metabolism of flatfish studied here invariably gave values falling between weight and surface proportionality. Considerable intraspecific variation in ( $b-1$ ) could not always be attributed to any specific environmental influence. However, as will be shown, important shifts in slope occurred concomitant with transfers of flounder to a changed osmotic gradient.

Considerable significance has been attached to the exponent  $b$  for a species in spite of recent reviews (124, 125, 126) pointing out the wide range of values that the slope,  $b$ , can take. Bertalanffy (12, 13) has categorized a large number of animals, including fish, into "metabolic types" depending on the proportionality (surface, weight, or intermediate) of metabolism. Notwithstanding numerous examples to the contrary (35) Bertalanffy has lumped fish as a group into one "metabolic type"—surface proportionality. The assumption made but not stated is that the slope neither changes during the ontogeny of the species nor is influenced by physiological alterations affected by environmental stress. Neither of these assumptions is tenable on the basis of experimental evidence.

Zeuthen (125) has presented several examples of animals which show significant changes in metabolic rate during their ontogeny. Fishes form no exception. Shamardina (107) found that metabolic rate of pike increased with growth of larvae, then inflected sharply and fell during postlarval, juvenile, and adult growth. The same author cites similar findings of three other Russian workers: Bezler working with carp and bream, Korzhuev

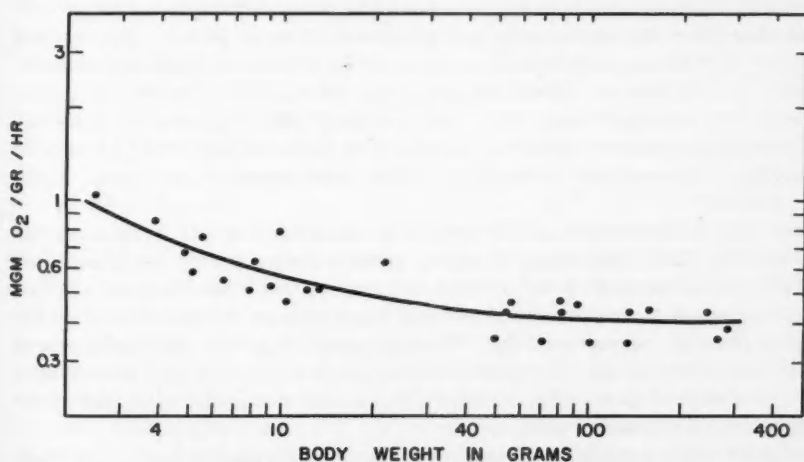


FIG. 4. Standard metabolic rate of winter *Platichthys stellatus* showing the departure from the linear log weight - log rate relationship seen in summer flounder. Line fitted by eye. Experimental temperature 10° C, salinity 24.7-26.0‰.

with sturgeon, and Privolnev with salmon. Lindroth (74) and Zeuthen (124) also report significant changes in slope during growth of fish. Therefore, it is impossible to neglect ontogenetic changes in the percentage decrease in metabolic rate with increasing body size of fish.

Usually no ontogenetic change in slope was apparent in the present studies on flounder and sole. All of the spring and summer determinations yielded data that conformed to a straight line on logarithmic paper. However, data collected on winter flounder measured at 10° C strongly suggest a percentage increase in slope towards weight proportionality with increasing body size. These data are shown in Fig. 4. Why such an effect should appear in the winter (10° C) but not in the summer (15° C) is difficult to understand, but it is possible that the percentage increase in slope reflects heightened metabolic demands of the large flounder with the approach of the spawning season (December through March). For purposes of statistical comparison of data collected with different salinity treatments, the points were assumed to conform to a linear log weight - log rate relationship with no change in slope.

Sometimes changes in slope were noted in comparing weight-rate curves obtained from flounder measured at the same salinity and temperature but at different times of the year. The results imply seasonal effects. Though seasonal influences or cycles were not a part of this work and were not studied, the few observations made show the need to account for seasonal effect before placing any significance in the slope of regression of metabolic rate.

#### *Diurnal Rhythm in the Metabolic Rate of Starry Flounder*

To assess the complete diurnal activity cycle, 14 flounder of all sizes, from 6.7 to 225 g, were simultaneously carried through one 24-hour period. After the initial 18-hour delay, oxygen consumption measurements were begun and continued for 24 hours, with measurements every 3 hours. During this period the fish were completely protected from external stimuli such as light, noise, and vibration. The temperature varied 0.1° C. The fish had been acclimated to temperature (15° C) and salinity (26.5‰), conditions closely approximating those in the environment from which the fish were captured at that time of year (late September). They were starved 4 days prior to the experiment.

In Fig. 5, the weight-specific oxygen consumptions of the 14 flounder are plotted for each of the eight sampling periods during the 24 hours and lines of best fit calculated by the method of least squares. The data were tested by analysis of covariance to determine whether true differences existed between the eight regression lines. The hypothesis that there are no differences between effect of the time periods is rejected at the 1% probability level ( $F = 3.18 > F_{.01} = 2.82$ ), indicating that the metabolic rate during the 24-hour period was not uniform.

Considerable variability is present around the regression line. The small flounder in particular show rather wide departures from the mean. Part of this variability is due to the error in the method because it was possible

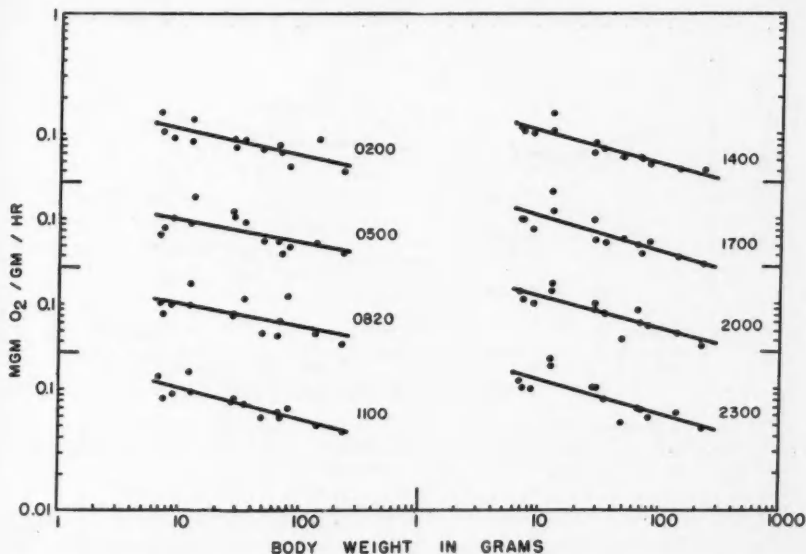


FIG. 5. Metabolic rates of 14 *Platichthys stellatus* measured at 3-hour intervals over one 24-hour period. Each point represents one determination for a fish at the indicated time of day. Lines are fitted by the method of least squares. Experimental temperature 15.0° C, salinity 26.6‰.

to analyze only one oxygen sample for each fish at each time period, whereas at least two and often three samples were collected for each fish in the salinity effect experiments and the results averaged. However, most of the variation is due to true physiological differences in the metabolic rates of the flounder themselves. A close examination of Fig. 5 shows that certain of the flounder in particular hold definite positions above or below the regression line. The 12.3-g flounder, for instance, would appear to have an inherently high total metabolism, the 7.1- and 81.6-g animals, inherently low metabolic rates. This sort of intrinsic variation is well known in lower vertebrates, is normal but troublesome, and necessitates using a rather large sample size for proper statistical treatment.

Figure 6 represents a summary of the experiment. From each regression line two points were taken, one representing a 10-g, the other a 100-g flounder, and plotted on a semilogarithmic grid. The cycle is illustrated best by the lower plot (for 100-g flounder) since less variability existed in the large than in the small fish. Oxygen consumption is highest at night. With the approach of daylight hours (though these experimental animals were maintained in darkness throughout) the rate of oxygen consumption declines and, at least for the large fish, reaches a low point in late afternoon before increasing sharply in the evening. The small flounder did not show a similar decline during the daylight hours, possibly because any true changes were masked by the large variability of the small fish.

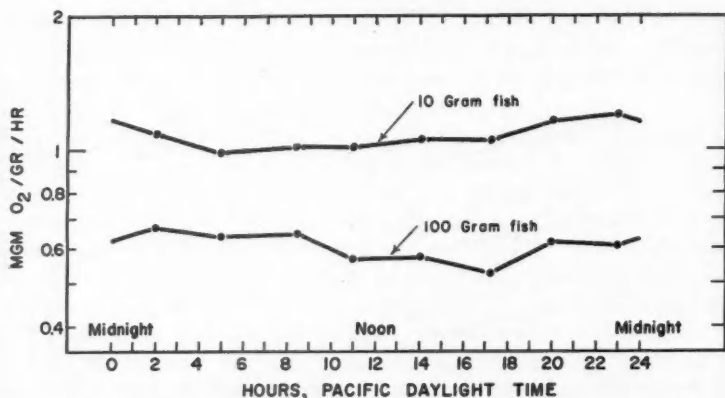


FIG. 6. Diurnal variations in metabolic rate of *Platichthys stellatus*. The "10-g fish" and "100-g fish" rates are derived from the points in Fig. 5 where the lines of best fit cross the 10-g and 100-g lines of each of the eight time periods.

#### *Effect of Starvation on the Standard Metabolic Rate of Starry Flounder*

Two experiments were performed to assess the effect of starvation on the rate of oxygen consumption. As with the previous experiment on diurnal rhythm, this experiment was not designed in any way as a definitive study of caloric starvation effects on metabolic rate but rather to evaluate the influence of this variable on the salinity-effect experiments to be presented in the next section. Both spring (May) and winter (December) fish were studied.

The oxygen consumption of a selected size range of spring flounder was determined on the 4th, 7th, and 20th days after collection. During this period, the flounder were held unfed under controlled conditions of temperature ( $15^{\circ}\text{C}$ ) and salinity ( $25 \pm 2$  ‰). As with all the metabolism experiments, the animals were introduced into the respirometers 20 hours before water analyses were begun. The three starvation periods were statistically treated by analysis of covariance two at a time to determine whether true decreases in oxygen consumption occurred. In both cases, the tests showed significant drops in standard metabolism: 4 days vs. 7 days starvation,  $F = 5.1 > F_{.05} = 4.16$ ; 7 days vs. 20 days starvation,  $F = 20.6 > F_{.01} = 7.4$ .

The temperature of the environmental locale of the winter fish collected in mid-December was  $10.5^{\circ}$ , thus the experiments were performed at  $10^{\circ}\text{C}$ . In other respects, experimental conditions were the same in both the winter and spring studies. Standard metabolic rate of a selected range of small- to medium-sized flounder was determined on the 2nd and 11th days of starvation. The adjusted means of regression of respiratory rate on body weight were compared by covariance analysis and the difference in the means found highly significant ( $F = 37.2 > F_{.01} = 7.31$ ).

Figure 7 shows the adjusted mean rates of oxygen consumption for each regression of summer and winter fish calculated from the covariance analyses by the formula ( $\text{adj. } \bar{Y}_i = \bar{Y}_i - b(\bar{X}_i - \bar{X})$ ). The drop in standard metabolic

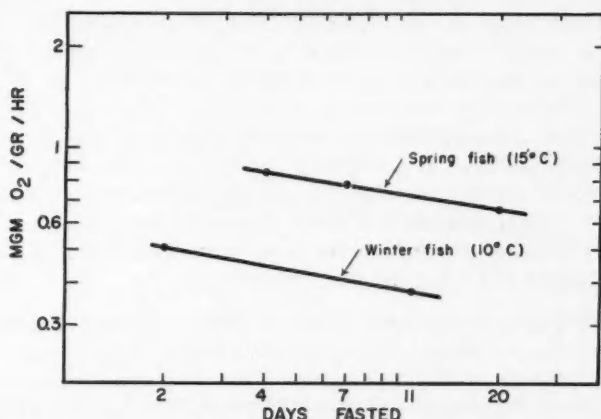


FIG. 7. Decrease in standard metabolic rate of *Platichthys stellatus* due to starvation.

rate of the spring fish is essentially logarithmic and the adjusted means of regression may be fitted with a straight line when plotted on a logarithmic grid. The means of the winter rates are included for comparison, though it can only be assumed that the drop is logarithmic because of the absence of a third time period in this group.

Since no fed controls were followed along with the fasted flounder, it is uncertain whether the systematic fall in respiratory rate is due entirely to starvation. A small part of the change might be caused by thermal acclimation during this period if discrepancies existed between the true environmental temperature and the laboratory temperature. Regardless of whether the drop in metabolic rate is due entirely to starvation or partially to starvation and partially to some other influence such as thermal acclimation, the drop will henceforth be referred to as a starvation effect. Data collected for the effect of salinity on metabolic rate was corrected for starvation by referring any two group comparisons to the same day of fasting. For instance, if the metabolic rates of fresh-water-adapted flounder fasted 4 days are being compared to a control group of sea-water flounder fasted 3 days, the individual metabolic rates of the 4-day-fasted group are divided by a fraction representing the difference between 3- and 4-day metabolic rates. This fraction was graphically derived from an enlarged graph of Fig. 7.

The question arises as to whether or not the depletion of stored energy reserves during fasting with the systematic fall in metabolic rate in any way interferes with normal osmotic exchange in flounder. There seems little doubt that sufficient energy exists for osmoregulation, for even the capacity to perform muscular work suffers little or no impairment. Barrett (unpublished, cited by Fry (35)) demonstrated that fasted *Salmo gairdnerii* can perform muscular work as well as fed controls and consume essentially as much oxygen in active metabolism experiments as did the controls. Many fish cease feeding entirely during spawning migration with no apparent

decrease in swimming ability. However, careful measurements have shown a progressive though small decrement in the swimming ability of fasting Pacific salmon as they migrate up the Columbia River (92). In this investigation the salinity effect experiments were carried out during the first few days of the fast. During this time, carbohydrates and fats supply nearly all of the energy needed. It is not until late in starvation, when combustion of tissue proteins commences, that it is possible to tax severely homeostatic mechanisms. For this reason, it seems a reasonably safe assumption that the few days of starvation imposed on the experimental flatfish had a completely insignificant effect on normal osmotic exchange.

*Effect of Salinity on the Standard Metabolic Rate of Starry Flounder*

Pilot experiments revealed that moderate changes in the osmotic gradient were inadequate to demonstrate differences in standard metabolism. Flounder transferred from hypertonic sea water to isotonic (14‰) or hypotonic sea water (e.g. 8‰) showed no significant change in metabolic rate.

Flounder introduced into fresh water showed a significant drop in metabolic rate. The decrease in oxygen consumption appeared to be gradual and became increasingly marked after the fish had adapted to fresh water. Figures 8 and 9 show that a decrease in standard metabolism has occurred in flounder 20 hours in fresh water as compared with sea water controls of the same nutritional state (8% decrease in the mean rate of oxygen consumption). The decrease is significant at the 10% but not at the 5% probability level ( $F_{.05} = 4.17 > F = 3.04 > F_{.10} = 2.88$ ). After 5 days in fresh water (Fig. 9), the standard metabolism of fresh- and salt-water flounder in the same nutritional state have diverged to a 10.5% difference in mean rates. This drop in metabolic rate (difference in the regression means) is highly significant ( $F = 11.06 > F_{.005} = 8.83$ ). These experiments were replicated in two successive years (summers of 1956 and 1957) with the same results: metabolic rate of flounder was consistently less in fresh water than in sea water.

The effect of adaptation time in fresh water is shown in Fig. 10. In the interval between 1 and 4 days in fresh water a highly significant drop in metabolic rate has occurred ( $F = 29.1 > F_{.01} = 7.88$ ). Both groups of fish were in the same nutritional state at the time metabolic rates were measured (6 days unfed at 15° C), hence the drop is due entirely to fresh-water adaptation.

The percentage decrease in metabolic rate of flounder transferred to fresh water is dependent on body size and is greater in small than in large animals. This observation is based on the decreases in slope of the regression line through the oxygen consumptions of fresh-water flounder as compared with their salt-water controls. The slope of the 20-hour-adapted fresh-water flounder (Fig. 8) is  $-.124$  and  $-.140$  for the control group. For the 5-day fresh-water-adapted flounder (Fig. 9) the slope is  $-.142$  as compared to  $-.192$  of the salt-water controls. The decrease is small but consistent and is in agreement with other experiments which sometimes showed greater decreases in slope.

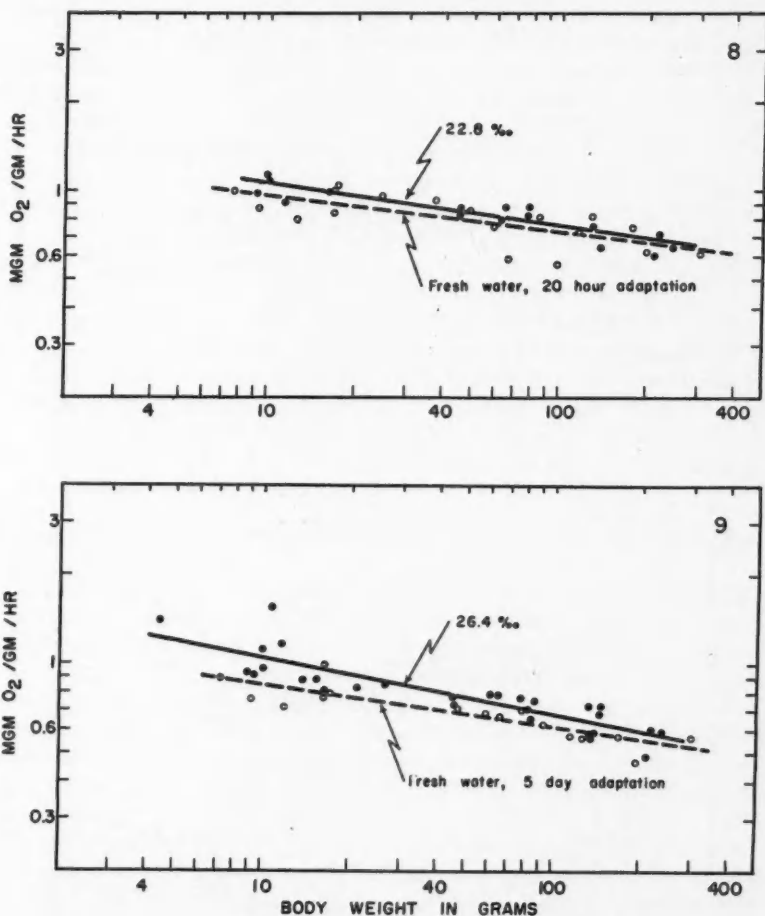


FIG. 8. Comparison of standard metabolic rate of *Platicthys stellatus* in 22.8‰ sea water (closed circles); and in fresh water, 20-hour adaptation (open circles). The regression lines are significantly different at the 10% probability level. Experimental temperature  $15.0 \pm 1^\circ \text{C}$ .

FIG. 9. Comparison of standard metabolic rate of *Platicthys stellatus* in 25‰ sea water (closed circles); and in fresh water, 5-day adaptation (open circles). The regression lines are significantly different at the 1% probability level. Experimental temperature  $14.8 \pm 1^\circ \text{C}$ .

The metabolic rate of flounder in concentrated sea water is significantly greater than the rate in normal sea water. An example is shown in Fig. 11. The mean standard metabolic rate of flounder in 43‰ is 15.1% above the mean of the control group. The difference is highly significant, with a variance ratio of 17.3. These results agree with a similar experiment carried out in the winter at  $10^\circ \text{C}$  though the mean percentage increase in metabolic

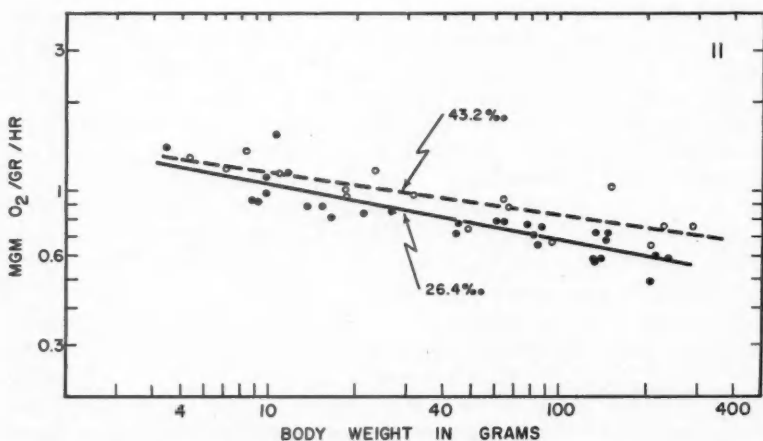
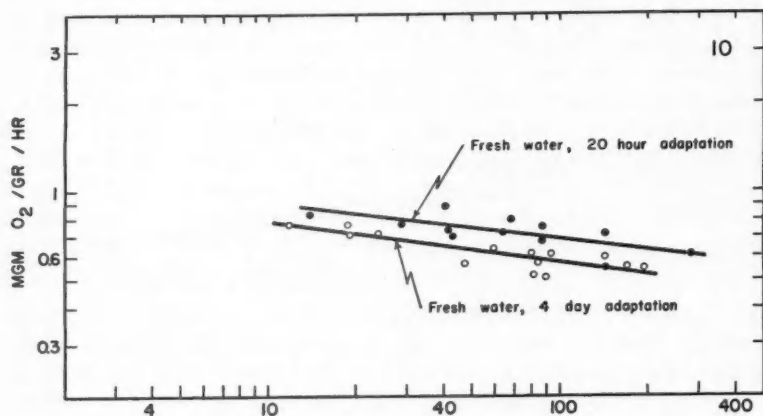


FIG. 10. Effect of adaptation time in fresh water on the standard metabolic rate of *Platichthys stellatus*; 20-hour adaptation rates are represented as closed circles and 4-day adaptation as open circles. The regression lines are significantly different at the 1% level of probability.

FIG. 11. Comparison of standard metabolic rate of *Platichthys stellatus* in 25‰ sea water (closed circles) and in 43.2‰ sea water (open circles). The regression lines are significantly different at the 1% probability level. Experimental temperature 14.0–14.8° C.

rate is not as great (15.1% in the summer experiment as opposed to a 25% increase in the winter). It may be pointed out that percentage increase in rates may not be the significant expression of these results since the osmotic work involved may not be proportional to the standard metabolic rate of the species. It is worth noting that the absolute increase in metabolic rate is nearly the same in the summer as in the winter group, 0.124 and 0.128 mg O<sub>2</sub>/g/hr respectively.

The slopes of regression, though different from the controls in both cases, are inconsistent in the direction of change and preclude any generalization as to a possible interaction between body size and salinity effect so far as the high salinity is concerned. In the spring experiment (Fig. 11), the slope is less than that of the control group ( $-.151$  and  $-.192$  respectively) and may indicate a relatively greater energy expenditure for osmotic regulation in the large than in the small animals. In the winter experiment, however, the slope became steeper in the high salt concentration ( $-.203$  at  $49\text{‰}$  and  $-.16$  at  $25\text{‰}$ ). Thus, these results, while unquestionably demonstrating a higher expenditure of energy in the high salt concentration for all fish, are inadequate with respect to showing whether the energy demands in the high salinity are relatively greater for small than large flounder, or vice versa. In fresh water, on the other hand, energy demands for osmoregulation are significantly less. The results as well show a consistent decrease in slope in the weight-specific oxygen consumption of flounder in fresh water, implying that osmoregulatory demands for energy are relatively less for small than large flounder in fresh water as compared with the energy expenditure of small and large salt-water flounder.

*Effect of Salinity on the Standard Metabolic Rate of Lemon Sole and Speckled Sand Dab*

Because an adequate supply of lemon sole (*Parophrys vetulus*) and speckled sand dab (*Citharichthys stigmaeus*) was unpredictable at best and actually unavailable most of the time, it was not possible to replicate completely with these species the salinity effect experiments carried out with flounder. Only scattered experiments were conducted when an adequate size range of either species was collected. The two experiments here presented, one on the sole, the other, the sand dab, are of some value as a comparison of the influence of salinity on the respiration of these stenohaline forms with the euryhaline starry flounder.

Figure 12 shows that an increase in the ambient salt concentration is accompanied by a marked increase in total metabolic rate of the speckled sand dab. The experiment was carried out after a 3-day adaptation period in concentrated sea water of  $43\text{‰}$  ( $\Delta 2.275^{\circ}\text{C}$ ) and is compared with a control group of the same nutritional state (fasted 3-4 days) in  $23\text{‰}$  sea water ( $\Delta 1.24^{\circ}\text{C}$ ). The difference in the means of regression is highly significant with a variance ratio of  $44.6$  ( $F_{.01} = 7.56$ ). There is also an increase in slope in the high salinity ( $b = -.203$  as compared with  $-.095$  for the controls). This suggests that the actual amount of energy expended for osmoregulation as a percentage of the total metabolic rate is influenced by body size, with a greater increase in energy expenditure for osmotic work in small than in large sand dab. This interpretation must be accepted with caution because it was not possible to replicate the experiment. It should be noted that the respiratory response of sand dab exposed to increased salinity is the same as that of starry flounder in increased salinity—an over-all increase in total metabolic rate.

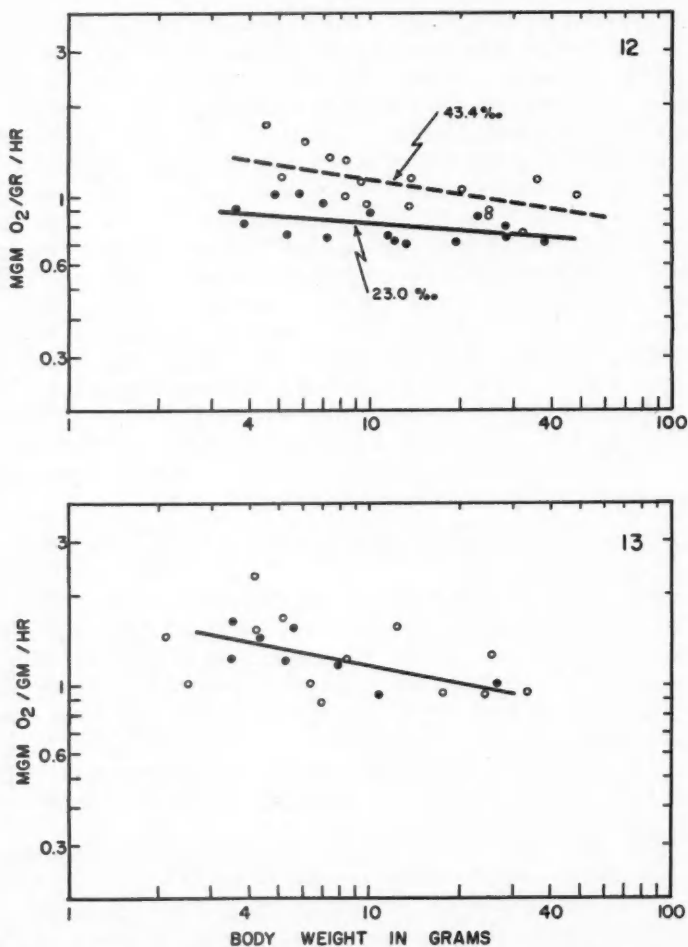


FIG. 12. Comparison of standard metabolic rate of *Citharichthys stigmaeus* in 23‰ sea water (closed circles) and in 43.4‰ sea water (open circles). The regression lines are significantly different at the 1% level of probability. Experimental temperature  $14.9 \pm .2^\circ \text{C}$ .

FIG. 13. Comparison of standard metabolic rate of *Parophrys vetulus* in 24.3‰ sea water (closed circles) and in 5.8‰ sea water (open circles). No significant change in metabolic rate occurred although individual variability was greatly increased in the group in the low salinity. Experimental temperature  $15.0 \pm .2^\circ \text{C}$ .

As pointed out in the introduction, the measurement of metabolic changes attending the transfer of a marine fish into a sea-water dilution below the incipient lethal salinity level has questionable physiological significance. Nevertheless, the procedure offers an interesting comparison between the metabolic responses of euryhaline and comparatively stenohaline marine

fishes introduced into low salinities. Such an experiment was performed and is illustrated in Fig. 13. The standard metabolic rates of a group of lemon sole were determined 3 days after transfer from normal sea water to a salinity of 5.8‰ ( $\Delta 0.31^\circ\text{C}$ ). The results show that essentially no change had occurred in the mean metabolic rate of these sole as compared with the controls, but that there was a large increase in individual variability. Lemon sole generally survived less than a week in 6‰ sea water. In this experiment some mortality occurred at the time of the oxygen consumption determinations; the attendant variability in metabolic rate reflects the moribund condition of the sole.

### Thyroid Activity and Radioiodide Metabolism

The frequently implicated role of the teleost thyroid in water and electrolyte metabolism has remained obscure. The problem has been approached both by measuring alterations in thyroid activity effected by salinity changes and by measuring changes in salinity tolerance induced by administering thyroid preparations. The literature on this subject has been reviewed by Fontaine (29,30), Hoar (49, 51), Smith (111), and Pickford and Atz (93). If a generalization can be ventured from the existing evidence, some of which is contradictory, it appears that thyroid activity decreases with increasing salinity of the external environment. Fresh-water species transferred to dilute sea water frequently show a transitory thyroid inactivation more noticeable in those species with particularly active glands in fresh water (86, 87). Conversely, marine species maintained in hypotonic salinities usually develop hyperactive thyroids. Hoar (50) found that landlocked smelt (*Osmerus mordax*) and alewives (*Pomolobus pseudoharengus*) always had more active thyroids than individuals collected from coastal estuaries. The landlocked alewives had extremely hyperplastic glands and experienced heavy mortality during the reproductive season when demands on thyroid hormone are evidently increased. Leloup (73) and Oliveau (85, 87) noted a transitory decrease in thyroid activity of two marine species, *Muraena helena* and *Labrus bergylla*, subjected to dilute sea water followed by a gradual return to normal activity in the lowered salinity. Transfer of the marine killifish, *Fundulus majalis*, from sea water of 25.5‰ to dilute sea water of 5.1‰ caused a small (25%) increase in thyroid uptake of  $\text{I}^{131}$  (39), while the same treatment with the brackish water *Fundulus heteroclitus* resulted in a very much greater (150%) increase in the peak  $\text{I}^{131}$  uptake of fish in the lower salinity.

The most convenient explanation for these findings is that fresh-water fish have a greater physiological demand for thyroid hormone in a direct or adjunctive osmoregulatory role. A more likely explanation, however, lies in the low iodine levels of fresh water as compared with sea water. Since Marine and Lenhart in 1910 (76) demonstrated that thyroid hyperplasia in brook trout, *Salvelinus fontinalis*, could be completely abolished by adding small amounts of iodine to the water, several authors have shown that thyroid

hyperactivity in fresh-water species is frequently a goitrogenic reaction to the low iodine content of fresh water. Both the uptake of radioiodine (7,8, 71) and histological criteria (45, 50, 70, 100, 104) have been used to demonstrate hyperplasia and to show that the condition was immediately alleviated by addition of even minute amounts of iodine to the water. Lack of iodine prevents normal production of thyroid hormone, resulting in a lowered titer of hormone in the blood. With the normal inhibiting action of thyroid hormone removed, more thyroid-stimulating hormone from the anterior pituitary is produced, resulting in a distention of the thyroid follicles with an incomplete form of thyroglobulin or "colloid".

Not all experimental findings can be explained on the basis of the iodine content of the water. Among the most interesting experiments are those of Koch and Heuts (65) and Heuts (48), who found that feeding thyroid to the euryhaline stickleback, *Pygosteus*, markedly decreased the resistance of this species to sea water. The treatment appeared to have a direct effect on mineral metabolism; chlorides accumulated in the blood, and the animals eventually died. Fresh-water sticklebacks were unaffected by thyroid feeding. Similarly, Baggerman (1) found that thyroxine administration induced a fresh-water preference and thiourea a salt-water preference in the three-spined stickleback, *Gasterosteus*. Often, however, interpretation is difficult because induced changes in salinity tolerance may be reversed by continued treatment.

The recent work of Burden (17) and Smith (111) strongly suggests that at least for the species studied, killifish and trout, an osmoregulatory role of the thyroid, if present, is collateral or subsidiary to some other endocrine influence. Smith's work with *Salmo trutta* showed that while thyroxine in high dosages promoted salinity resistance, growth hormone was far more effective in this respect. Burden's work indicates the involvement of an unknown pituitary factor in osmoregulation of *Fundulus*. Thyroxine therapy of hypophysectomized fish had no effect on the inability of these animals to survive in fresh water. Gorbman (in discussion following paper by Smith (111)) found that thiourea treatment had no effect on the normal euryhalinity of *Fundulus heteroclitus*. Treated fish with inhibited thyroid glands resisted transfer to either fresh or salt water as well as controls.

It is doubtful whether the administration of thyroid material or the inhibition of thyroid function with chemical inhibitors are ideally effective ways to demonstrate an osmoregulatory role of the thyroid gland. One criticism is that any therapy may have unknown side-effects that could alter the normal physiology of the species. Until fundamental research, presently lacking, reveals the effects of thyroid inhibitors, such as thiourea or thiouracil, on all aspects of the fishes metabolism, they should be used with caution. Similarly the feeding or injection of thyroid substance may have far-reaching effects not immediately apparent. For one thing, thyroid administration inhibits secretion of TSH resulting in a marked hypoactivity of the animal's own thyroid gland. Another argument against such treatment is that it is difficult to know when or whether the treatment has been effective.

As already mentioned, the apparent effect induced by treatment may actually reverse if the treatment is continued long enough.

Several criteria for evaluating thyroid activity are available, but only two, histological and radiological, have been used extensively by fish physiologists. Of the radiological methods, the simple uptake of radioiodine by the thyroid gland has become a research tool of considerable importance. Tests usually attempt to determine the amount of radioiodine trapped by the thyroid by measuring with counting instruments the proportion of the dose accumulated. In practice, a large number of fish must be given a standard dose simultaneously, sacrificed at intervals of time thereafter, the thyroids removed, and their radioactivity counted. The percentage thyroid uptakes of individual fish are plotted against time of sacrifice to form a composite uptake curve. Injections are usually made intraperitoneally. These curves appear in most cases to be a reliable estimate of the true thyroid activity (the rate of secretion of thyroid hormone) under many experimental conditions. Three very important factors, however, limit the diagnostic accuracy of radioiodine tracer studies in fishes. One is the amount of elemental iodide in the ambient water as already mentioned. A second is the variable amount of thyroid tissue in the head kidney of some fish such as goldfish (19). A third important factor is the disappearance rate of the tracer dose from the blood. It is clear that collection of radioiodine by the thyroid gland is totally dependent on the amount of isotope delivered to the gland by the blood stream. Other things equal, less  $I^{131}$  will accumulate in the thyroid when the injected dose is rapidly removed from the body (resulting in a rapid drop in  $I^{131}$  concentration in the iodide space) than when removal is slower. If the excretion rates of radioiodine differ under different experimental conditions, it may not be justifiable to use thyroid  $I^{131}$  uptake curves as a comparison of thyroid activity between experimental treatments. Salinity variations particularly would be expected to produce changes in iodide behavior in the body fluids because of the marked quantitative and directional alterations in electrolyte and water movements across the organs of exchange: the kidney, gills, oral membranes, and alimentary canal. Temperature variations should also influence iodide excretion rates, since metabolic rate and concomitantly the rate of electrolyte exchange of poikilothermic animals such as fish are directly dependent on the environmental temperature. For those reasons it must be concluded that thyroid  $I^{131}$  uptake in itself is an unreliable parameter for evaluating thyroid activity if either salinity or temperature are altered as experimental treatments.

To evaluate thyroid activity of flounder in fresh and salt water, another parameter, that of thyroid clearance has been chosen. Direct and indirect measurements of thyroidal clearance of radioiodide from the blood has frequently been used as a precise measure of the iodine-accumulating activity of the mammalian thyroid (99, 105). Although it is generally not used clinically because it is difficult to perform, thyroid clearance is one of the most accurate diagnostic tests of clinical severity of hyperthyroidism in man (16, 106). Thyroid clearance appeared ideal for the present investigation

with flounder since it satisfies the requirement for a test that takes into account variations in the disappearance rate of radioactive iodine from the blood.

Thyroidal radioiodide clearance, expressed as the volume of blood cleared of its radioiodide per minute, is a modification of the standard renal clearance formula:

$$I^{131} \text{ clearance, thyroid} = \frac{\text{thyroid } I^{131} \text{ uptake during } t \text{ minutes}}{\text{mean blood concn. of } I^{131} \text{ during } t \text{ minutes}}.$$

In the present study, thyroid activity of the starry flounder as influenced by salinity has been studied with radioiodine, paying particular attention to the behavior of the tracer dose in the body fluids. With the exception of some preliminary work by Chavin (20) essentially nothing is known of iodine metabolism in fishes. A large portion of the research was devoted, therefore, to radioiodine movements—its excretion, distribution in the body, and behavior in the blood—in both fresh- and salt-water flounder. In comparing thyroid activity in fresh and salt water, flounder were preadapted to fresh water containing an added amount of iodide equivalent to the iodine content of sea water at the experimental salinity used. As with the foregoing sections on body fluid concentration and metabolic rate, attention has been given to the effect of size on thyroid activity.

#### DETERMINATION OF THYROID ACTIVITY WITH RADIOIODINE

Thyroid clearance in flounder was measured in two ways. The first method was to measure the radioactivity of thyroid and blood samples removed from individual fish sacrificed at intervals after injection. This method had the advantage of simplicity of procedure and high counting accuracy but required large numbers of fish. The second method used was serial *in vivo* counting of thyroid radioiodine uptake with a collimated scintillation counter together with serial sampling of blood from the caudal circulation. In this way a great deal of information was gained from each fish, and measurements could be continued for long periods until radioactivity was too low to count with any accuracy.

#### *Interval Sampling*

Radioiodine in the form of carrier-free sodium iodide was diluted with saline or distilled water for injection. For any one experimental series, the dosage of  $I^{131}$  injected into each fish was the same, usually 5 microcuries ( $\mu\text{c}$ ) per fish. The volume of injected fluid containing the  $I^{131}$  was always 0.05 ml per fish. Injections were made intraperitoneally with a 0.25-ml tuberculin syringe and 27-gauge needle. To prevent leakage of the injected fluid from the needle puncture, the flounder were injected from the blind side by passing the needle at an acute angle through the ventral musculature and into the posterior portion of the coelom. The muscle thus acted as a seal against fluid leakage after withdrawal of the needle.

Aliquots of each dose were reserved as standards and given appropriate dilutions with slightly alkaline water for counting with the samples.

As with most teleosts, the thyroid of the flounder is a diffuse gland with thyroid follicles scattered widely about the ventral aortae. By sectioning this area of the lower jaw of flounder previously injected with  $I^{131}$  and counting the radioactivity of individual small portions, it was determined that the thyroid always lay anterior to the third branchial arch with some extension of follicles a short distance laterally along the gill bars. Hence, in collecting thyroid samples as much nonthyroidal tissue as possible was trimmed from the lower jaw area without infringing on the region where thyroidal tissue was known to lie.

An end-probe scintillation counter with a 45 mm diameter  $\times$  38.5 mm thick NaI (TI) crystal<sup>5</sup> was used as the counting instrument for all studies except those associated with body size effect on thyroid activity. Total sample digestion was unnecessary with this counter, provided the samples and standards were similar in mass and geometry. Thyroid samples were placed directly into steel planchets (25 mm  $\times$  7 mm) with about 1 ml of hot 2 *N* NaOH and allowed to set overnight in a warm oven. This simple treatment effectively digested the tissue to spread the radioactivity evenly over the planchet. Standards representing 1/100 dose were prepared and allowed to spread evenly over the planchet bottom. Counting was carried out with the crystal 2 cm to 6 cm distant from the samples.

Most of the studies associated with body size effect on thyroid activity were carried out using a well-crystal scintillation counter.<sup>6</sup> Thyroid samples were collected, trimmed, and ashed in sodium hydroxide as before. One-milliliter aliquots of the samples were pipetted into 5-ml plastic vials ("Clearsite", 1½-dram size) and diluted to 4 ml with water. Counting was then accomplished with the scintillation well counter (38.5 mm  $\times$  51 mm NaI (TI) crystal) and a predetermined count scaler.

Uptake of  $I^{131}$  by the thyroid gland is expressed as percentage of dose of  $I^{131}$  accumulated by the gland per unit of time after injection. In all cases, the percentage uptake of the whole gland was measured, hence all flounder for any one experiment received a standard dose of  $I^{131}$  regardless of body weight.

The actual weight of the trimmed thyroid tissue as a percentage of the body weight varied considerably, but there appeared to be no unconscious tendency toward more thorough trimming in small or in large fish. The average weight of the trimmed tissue was about 1.3% of the total body weight and varied between 0.935% and 1.535% of the body weight.

Blood was collected at the time each flounder was killed by puncturing the dorsal aorta above the coelom and withdrawing the blood with a clean pipette as it welled up from the wound. The blood was placed in a steel

<sup>5</sup>The end-probe scintillation counter was made available through the generosity of Dr. Harold Copp and Dr. Carl F. Cramer of the University of British Columbia Department of Physiology.

<sup>6</sup>The scintillation well counter was made available by the British Columbia Medical Research Institute through the kindness of Dr. Peter Solvonuk.

planchet with Parafilm cover tared to four decimal places, and the cover firmly folded over the planchet top to prevent evaporation from the blood. The sample was then weighed, the Parafilm cover discarded, and 1 ml 2 *N* NaOH added to the blood in the planchet to dissolve the clot and spread the radioactivity evenly over the planchet bottom. After they were dried, samples and standards representing 1/100 dose were counted with the end-probe scintillation counter with the crystal 0.5 to 6 cm distant depending on the activity of the sample.

Urine samples were collected at irregular intervals by placing a long, thin pipette against the urinary papillae and gently pressing the body wall over the urinary bladder. The amount collected varied considerably between 0.1 and 1% of the body weight. The sample was placed into a tared, Parafilm-covered planchet, sealed, weighed, and counted as described for blood samples.

After blood and urine samples were collected the thyroid gland was removed, the body of each flounder was placed in a plastic petri dish (90 mm in diameter with 13-mm sides) and covered. A flounder too large to fit directly was cut into pieces and fitted into the dish, or, in the case of a very large flounder, divided among two or three dishes. Standards containing the entire dose were prepared either by injecting fish of representative sizes with the standard dose and immediately killing and placing them in petri dishes or by cutting layers of filter paper to represent roughly the shape of the fish, and adding the standard  $I^{131}$  dose to the paper in the petri dish. Bodies and standards of similar mass and geometry were then counted in the end-probe scintillation counter with the crystal 18 cm from the samples.

The amount of radioactivity in the body is conveniently expressed as percentage of dose remaining. The expression of blood and urine activity presents a different sort of problem because we are no longer dealing with entire organs or glands. A milliliter blood sample from a 10-g flounder would contain 10 times the percentage of dose as a milliliter blood sample from a 100-g flounder since both fish are given the same dose. Thus the body weight must be taken into account. This is done by multiplying the percentage of dose per gram of blood or urine by the body weight in grams: the "biological concentration coefficient" (22). In this study, the concentration coefficient is divided by 100 to give a value more easily compared to thyroid uptake values.

The calculation of "excretory" clearance (representing all routes of  $I^{131}$  excretion) and thyroidal clearance are simple modifications of the standard renal clearance formula, usually expressed as:

$$\frac{\text{concentration of A in the urine} \times \text{volume of urine}}{\text{concentration of A in the blood}}$$

or

$$\frac{\text{rate of excretion of A}}{\text{concentration of A in the blood}}$$

Substance A in this case is  $I^{131}$  and the formula becomes:

$$I^{131} \text{ clearance, excretory} = \frac{\text{rate of } I^{131} \text{ excretion during } t \text{ minutes}}{\text{mean blood concentration of } I^{131} \text{ during } t \text{ minutes}}$$

and

$$I^{131} \text{ clearance, thyroid} = \frac{\text{rate of thyroid } I^{131} \text{ uptake during } t \text{ minutes}}{\text{mean blood concentration of } I^{131} \text{ during } t \text{ minutes}}$$

$I^{131}$  is disappearing exponentially from the blood and its mean concentration may be calculated graphically by plotting  $I^{131}$  concentration on a log scale against time on a linear scale, or by the formula:

$$\bar{B} = \frac{B_1 - B_2}{\ln B_1 - \ln B_2}$$

where  $B$  is the concentration of  $I^{131}$  in percentage of dose (concentration coefficient) in the blood. Mammalian thyroïdal and renal clearance of  $I^{131}$  is discussed by Myant, Pochin, and Goldie (82), Berkson *et al.* (10), Keating *et al.* (60), Berson *et al.* (11), and Riggs (99).

#### *Serial in vivo Counting*

Serial *in vivo* counts of thyroid  $I^{131}$  uptake in flounder were made by collimating radiation from the area of the lower jaw. The instrument was a scintillation counter with a 38.5×45 mm NaI crystal, surrounded by 51 mm of lead and placed in a stand with the crystal facing up. This was covered with a 51-mm thick lead block provided with interchangeable plugs with 13-mm or 19-mm apertures. Medium- and large-sized flounder (70 to 350 g) were injected with radioiodine as described for interval sampling except that larger doses of radioiodine (20 to 30  $\mu$ c) were used to ensure adequate radioactivity of the small blood samples for counting. At intervals after injection, the unanesthetized flounder was placed on a Lucite tray, covered with a wet paper towel, and positioned over the lead block with the area of the thyroid directly over the collimator. The most satisfactory method of counting was to locate with a count rate meter the area of the head emitting the greatest radioactivity. Counts were then made directly with the rate meter or by switching the circuit to a scaler.

The rate of disappearance of radioiodine from the body was determined by counting the activity of the whole body at intervals after injection. After thyroid activity had been counted, the collimator block was removed and the fish was placed on a wooden stand 53 cm above the probe, and the body counted. At this distance, geometrical errors due to changing distribution of the tracer dose in the body were essentially eliminated. The entire procedure of counting thyroid and body activity occupied 1 to 2 minutes. The flounder never struggled during this period if covered closely with a wet paper towel.

Serial sampling of blood was carried out by direct needle puncture of the caudal artery with a 0.25-ml syringe and 27-gauge needle. With some practice, the artery could be readily located by introducing the needle into the caudal hypaxial musculature just ventral to the lateral line and passing the needle

inward and forward until the circulation was entered. A very small sample of blood (0.03 to 0.1 ml) was withdrawn, transferred to a tared Parafilm-covered planchet, weighed, and digested with NaOH. Dried samples and standard were counted with the end-probe scintillation counter.

Of the total collimated radiation detected by the scintillation counter from the area of the thyroid gland, a certain amount is emitted from extrathyroidal tissue, mostly blood and extracellular fluid. Many hours after injection this extrathyroidal activity is not important compared to thyroidal activity but during the first hours it is much greater by comparison and falling rapidly. Correction for extrathyroidal tissue was accomplished satisfactorily by determining the rate of disappearance of radioiodine from the nonthyroidal tissues of the body, correcting the total thyroid counts for this exponential fall in activity, and then converting the corrected counts to per cent dose. This procedure is illustrated in Fig. 14.

Multiple body and collimated thyroid counts were measured on the living flounder at intervals of time after injection of  $30\text{ }\mu\text{C}$  of carrier-free radioiodine. At termination, the thyroid is removed, trimmed, and its activity determined by counting with the thyroid positioned over the collimated scintillation counter exactly as it was in the intact fish. This portion of the total count is referred to as thyroidal, the rest as extrathyroidal. In the example shown (Fig. 14), the total thyroid count (last count on the living flounder) = 40,740 counts/minute (corrected for isotope decay), the thyroidal portion = 35,370

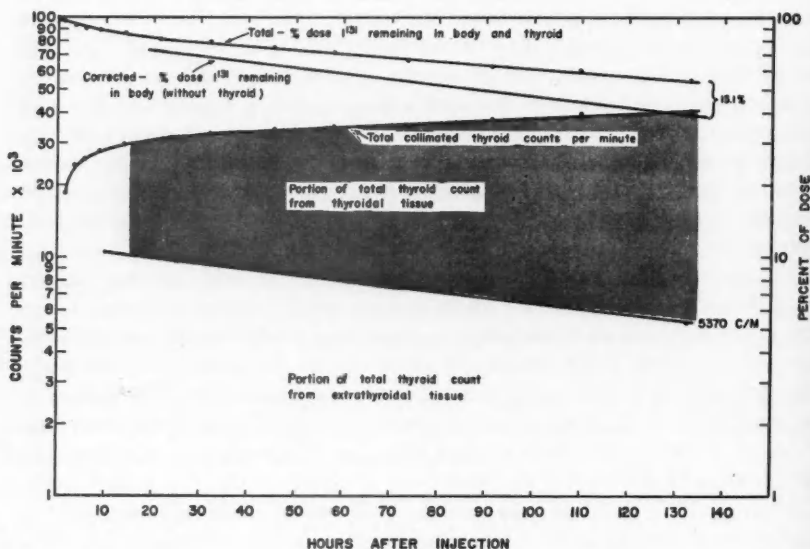


FIG. 14. Method of correcting total collimated thyroid counts for radioactivity emitted from extrathyroidal tissue surrounding the thyroid gland. See text for explanation.

counts/minute, and the extrathyroidal portion by subtraction = 5370 counts/minute. The thyroidal tissue is then digested and its activity ascertained precisely as per cent dose by reference to a standard.

The rate of disappearance of radioiodine from extrathyroidal tissue is derived from the disappearance rate of radioiodine from the body. Body counts are plotted as percentage of dose. In the example shown, the body at termination still held 54.4% of the initial dose, 15.1% of which was in the thyroid. The remainder (39.9%) represents the per cent of dose in the extrathyroidal tissue of the body. From this point a corrected slope is drawn back to near zero time. This slope, representing extrathyroidal tissue radioiodine removal rate, is transferred as shown to form the division between the thyroidal and extrathyroidal portions of the total thyroid count. Finally the corrected thyroid count is determined graphically and converted to per cent dose by proportion to the per cent of dose in the thyroid at termination.

## RESULTS

### *Factors Influencing the Excretion of Radioiodide*

#### *The Negligible Influence of Radioisotope Re-entry on Radioiodine Excretion*

Fish injected with radioiodine excrete the isotope directly into the environment in which they live. Unless the water is continually renewed, the concentration of radioiodine will increase in the ambient media and re-enter the body of the fish. It was not possible to keep flounder in constantly renewed water, since experimental treatment necessitated controlling the salinity, temperature, and elemental iodine content of the water. It was necessary therefore to determine whether isotope re-entry could influence thyroid radioiodine uptake.

Two groups of three flounder each were maintained in separate aquaria, each containing 7.5 liters of water, for a period of 80 hours under the same conditions of salinity (19 ‰) and temperature ( $18.0 \pm 3^\circ \text{C}$ ). After injection of the first group of fish, the water was changed at 2- to 3-hour intervals to prevent any accumulation of excreted  $\text{I}^{131}$  in the water. Water was never changed in the second group of flounder. The disappearance of radioiodine from the bodies was followed by measuring the radioactivity of the entire living animal at intervals with an end-probe scintillation counter. The results are shown in Fig. 15. Although the composite curves (inset) show a less rapid excretion for the re-entry group, the curves are not significantly different. In none of the experiments to be described were less than 2 liters of water per fish provided; usually the experimental tank contained 3 to 5 liters or more per fish.

Since the water in all experimental tanks was changed at least every 48 hours it is concluded that re-entry of excreted  $\text{I}^{131}$  was without effect on the behavior of the tracer dose in the thyroid and body fluids.

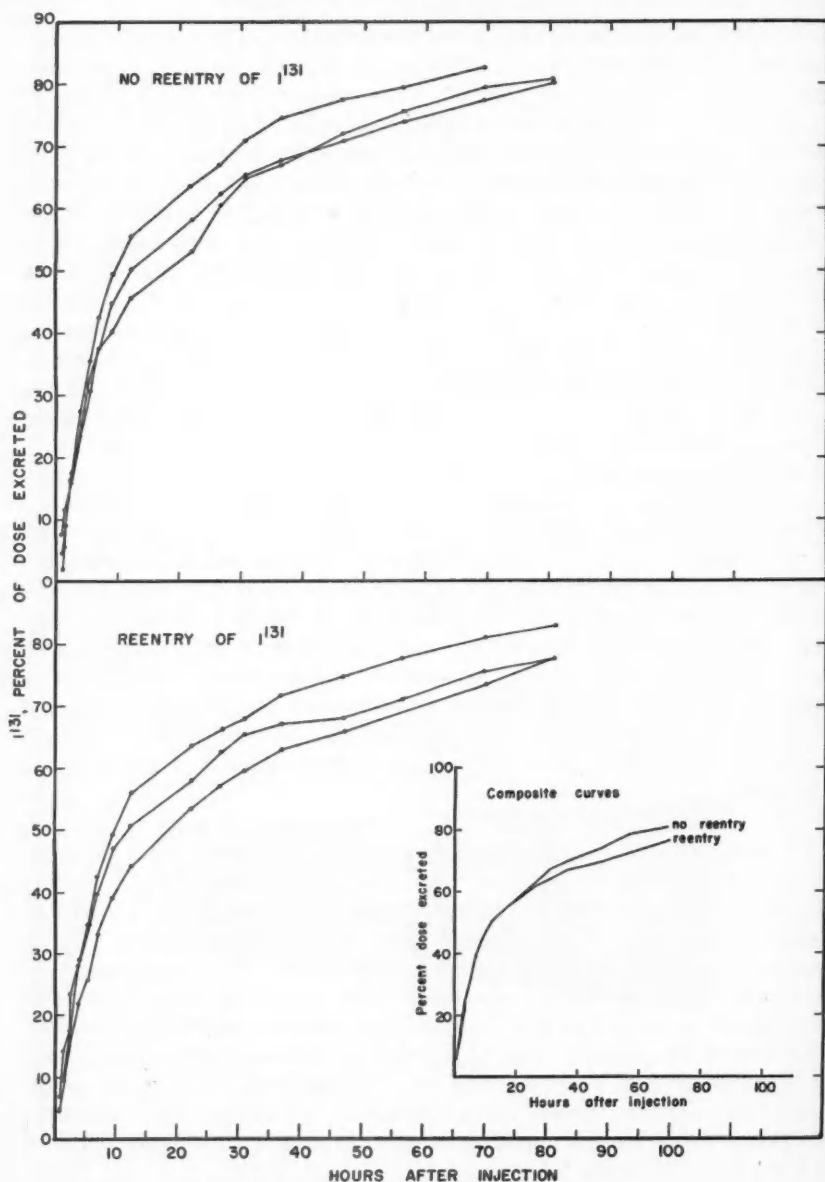


FIG. 15. Effect of re-entry into the body of excreted isotope on the radioiodine excretion curves. Individual curves show the total excretion of a single dose of radioiodine from the bodies of six flounder (22.9 to 48.6 g body weight). The three flounder shown by the upper curves were maintained in continually renewed sea water. The other three flounder shown in the lower curves were kept in a small volume of unrenewed water. The composite curves are not significantly different.

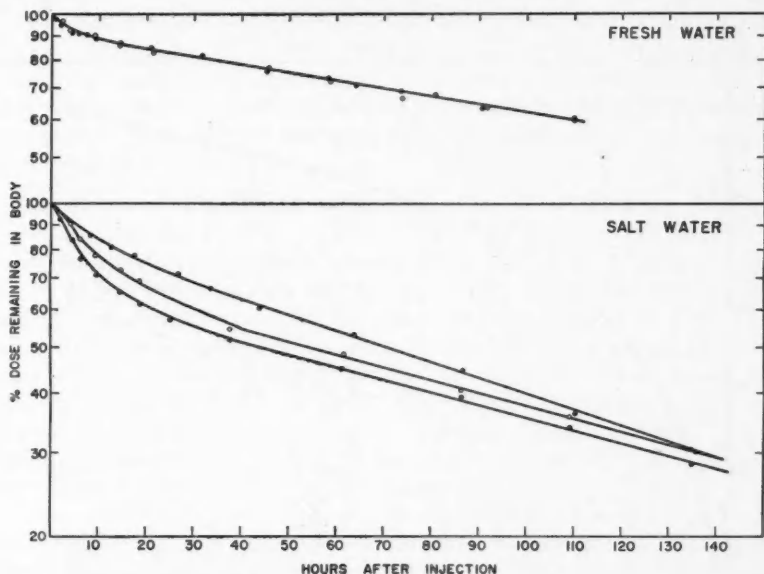


FIG. 16. Disappearance of radioiodine from the bodies of fresh-water and salt-water flounder. Individual *in vivo* measurements are shown by circles. Two fresh-water fish are represented (open and closed circles) with lines of best fit superimposed.

#### *The Exponential Removal of Radioiodine from the Bodies of Fresh- and Salt-Water Flounder*

After injection of radioiodine, there follows a variable period of absorption from the coelom and distribution of the tracer dose throughout the body fluids until equilibrium is attained. After equilibrium, radioiodide is removed from the iodide space by the pathways of excretion and thyroid accumulation at a rate that is proportional to the amount of tracer present. Figure 16 shows that after 10 to 35 hours radioiodine is excreted from the bodies of both fresh- and salt-water fish exponentially or at a constant fractional rate of change.<sup>7</sup> Equilibrium is reached in fresh-water flounder about 15 hours after injection (at 18–20° C). Before 10–15 hours, the iodide space is constantly enlarging so that the rate of excretion is initially high when the dose is concentrated in a smaller compartment. Absorption from the coelom occurs rapidly as indicated by the high blood radioiodine concentration during the first hours. A much longer period, up to 35 hours, is required before equilibrium is attained in salt-water flounder.

<sup>7</sup>The rate-constant  $k$  is the fractional rate of change of  $I^{131}$  with time and is calculated by the equation  $I^* = I_0^* e^{-kt}$  or  $k = 1/t (\ln (I_1^*/I_0^*))$  where  $I_1^*$  = amount of  $I^{131}$  present at the time  $t$  and  $I_0^*$  = amount of  $I^{131}$  present at zero time. The half-value time,  $t_{1/2}$ , representing the time for removal of half the  $I^{131}$  present is calculated by:

$$t_{1/2} = \frac{2.3 \log 2}{k} = \frac{0.693}{k}.$$

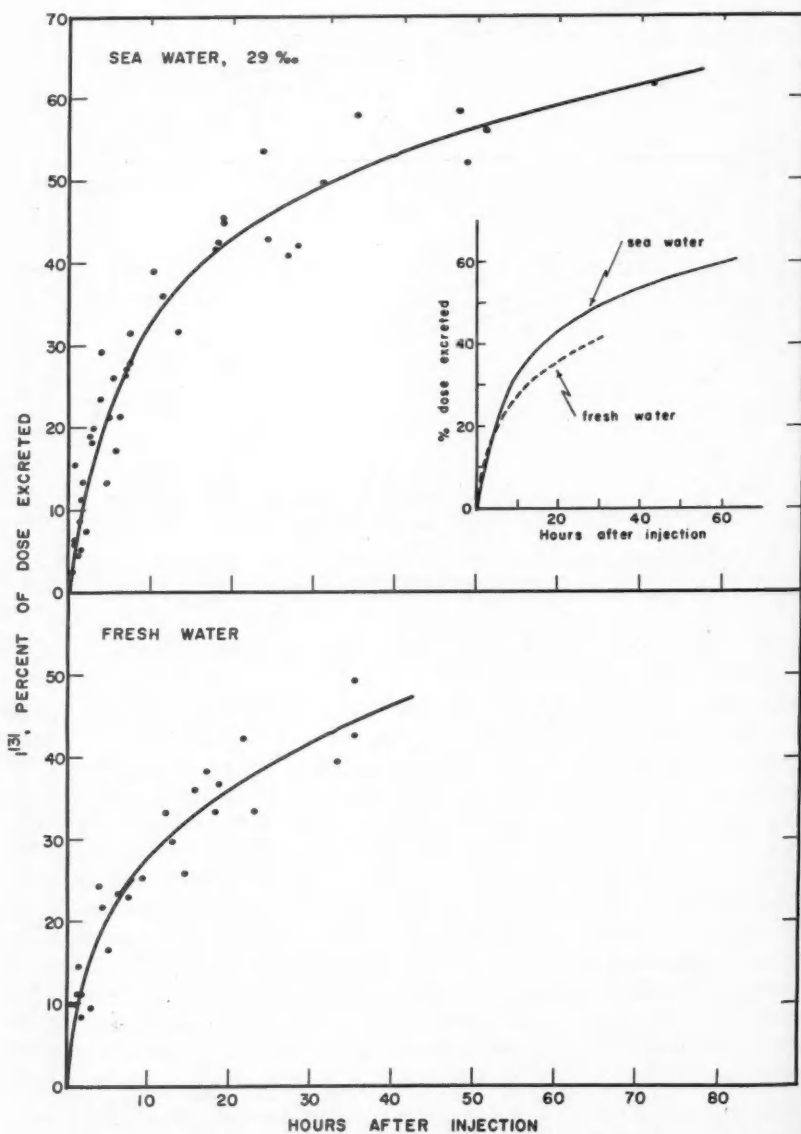


FIG. 17. Effect of salinity on the cumulative total excretion of radioiodine by *Platicthys stellatus*. Each point represents the proportion of a standard dose of radioiodine excreted by an individual fish.

The long pre-equilibrium period is also seen in the blood  $I^{131}$  concentration curves for flounder (Fig. 21). A logarithmic or near-logarithmic fall in concentration is not apparent until 15–40 hours after injection. However, the removal of  $I^{131}$  from the blood may never become exponential because of the gradual appearance of small quantities of hormonal-bound radioiodine in the circulation.

*The Effect of Salinity and Iodide Content of Fresh Water on Radioiodine Excretion Rate*

Radioiodine is excreted more rapidly in salt water than in either natural or iodine-enriched fresh water. Comparison of the cumulative total excretion of  $I^{131}$  by small fresh-water and salt-water flounder is shown in Fig. 17. The fresh-water group were given a 5-day preadaptation period in fresh water with added elemental iodine equivalent to the amount present in 30‰ sea water, 43  $\mu\text{g}$  iodine per liter. Both groups, therefore, were in water differing in total salt content, but of the same iodine content. Initially the fresh-water flounder lose  $I^{131}$  more rapidly but after the first few hours total excretion is more rapid in the salt-water group.

After the iodide space stabilizes and the excretion of radioiodine becomes exponential, it is convenient to use the rate-constant  $k$  and the half-value time ( $t_{1/2}$ ) to describe the disappearance rate. In Table III are given the body  $I^{131}$  disappearance rates measured in vivo for a number of starry flounder in salt water and in fresh water with and without the addition of elemental iodide.

The total excretion of radioiodine from the body (columns 1 and 2) of salt-water flounder has an average half-value of 106.7 hours. In fresh water, excretion is slower ( $t_{1/2} = 146.8$  hours) while the addition of iodine slows removal even further ( $t_{1/2} = 165.4$  hours). These values are somewhat misleading because the thyroid is trapping radioiodine in variable amounts and removing it from the iodide pool available for excretion. Values more representative of the disappearance rate of radioiodine are obtained by calculating the percentage of dose present in the thyroid and correcting the body values as explained under Methods. A curve drawn through these points represents the disappearance of radioiodine from the nonthyroidal tissue of the body. Removal rates calculated from these corrected curves are given in columns 3 and 4 of Table III.

The discrepancy between radioiodine removal rates of flounder in iodine-deficient fresh water and in iodine-enriched fresh water is greatly increased when corrected for thyroidal uptake. The difference lies in the greater thyroid radioiodine accumulation of flounder in fresh water of low iodine content. The half-value time for radioiodine removal from the body without thyroid (extrathyroidal tissue) averages 110.6 hours for flounder in natural fresh water and 151.6 hours for flounder in iodine-enriched fresh water. These averages are significantly different at the 5% level of probability ( $t = -3.42 > t_{.05(6)} = -2.45$ ). It should be emphasized that these rates describe radioiodine removal and not iodine removal. Specific activity, defined here as the

TABLE III

Disappearance rate of radioiodine from the bodies of starry flounder. All rates are calculated after removal of radioiodine from the bodies becomes exponential. Experimental temperature 17-18° C

( $k$  = rate constant representing the fractional rate of change of radioiodine concentration with time after injection)

$t_{1/2}$  = half-value time representing the number of hours for disappearance of half the radioiodine present)

| Disappearance rate<br>from entire body |                | Disappearance rate from<br>body without thyroid<br>(corrected slopes) |                | Body<br>weight, g |
|--|----------------|---|----------------|-------------------|
| $k$                                    | $t_{1/2}$ , hr | $k$   | $t_{1/2}$ , hr |                   |
| Salt water 23 ‰                        |                |   |                |                   |
| -.00785                                | 88.3           | -.00891   | 77.7           | 84.2              |
| -.00619                                | 111.8          | -.00751   | 92.3           | 123.9             |
| -.00676                                | 102.5          | -.00914   | 75.8           | 90.5              |
| -.00648                                | 106.9          |   |                | 38.3              |
| -.00576                                | 120.3          | -.00740   | 93.6           | 80.7              |
| -.00627                                | 110.5          | -.00770   | 90.0           | 74.2              |
| -.00655                                | 106.71         | -.00813   | 85.9           |                   |
| Natural fresh water                    |                |   |                |                   |
| -.004913                               | 141.0          | -.00602   | 115.2          | 73.8              |
| -.00401                                | 172.8          | -.00556   | 124.6          | 94.2              |
| -.00395                                | 175.2          | -.00545   | 110.7          | 79.8              |
| -.00605                                | 114.5          | -.00701   | 98.9           | 46.4              |
| -.00530                                | 130.7          | -.00669   | 103.6          | 88.3              |
| -.00484                                | 146.84         | -.00615   | 110.6          |                   |
| Iodine-enriched fresh water            |                |   |                |                   |
| -.00421                                | 164.6          | -.00482   | 143.7          | 103.7             |
| -.00489                                | 141.7          | -.00492   | 140.8          | 55.7              |
| -.00365                                | 189.8          | -.00407   | 170.4          | 67.1              |
| -.00425                                | 165.4          | -.00460   | 151.6          |                   |

percentage of dose of radioiodine per milligram of iodine in any chemical form, is presumably much less in flounder in iodine-reinforced fresh water than in iodine-deficient fresh water. In other words, a much smaller proportion of iodine atoms in flounder in iodine-enriched water are tagged, with the result that radioiodine excretion cannot be used as an index of iodine turnover unless absolute iodine concentration is also measured.

When environmental iodine concentration is the same, as in salt water and iodine-enriched fresh water, radioiodine turnover should be a reliable index of iodine turnover. Here the limitation imposed is that all fish be in iodine balance, i.e., that iodine uptake equal iodine excretion. Removal rates of radioiodine from nonthyroidal tissue differed greatly between these groups, with average half-value times of 85.9 hours for sea-water flounder as compared to 151.6 hours for flounder in fresh water with iodine content equivalent to that of sea water. These averages are significantly different at the 1% level of probability.

*The Predominance of Extrarenal Pathways for the Excretion of Radioiodine in Both Fresh- and Salt-Water Flounder*

Direct data are not available to indicate the relative importance of the several possible pathways of exchange of iodide with the external environment in either fresh or salt water. However, it is possible to make a rough estimate of the volume of urine needed to remove all of the  $I^{131}$  being excreted assuming that there is no extrarenal excretion of any kind. By comparing these hypothetical urine flows with average urine flow values for fresh- and salt-water species reported in the literature, one arrives at a rough indication of the proportion of  $I^{131}$  removed by renal and extrarenal pathways. Urine flows were calculated by dividing the proportion of the dose excreted per hour by the urinary concentration of  $I^{131}$  expressed as the biological concentration coefficient (% dose per gram of urine  $\times$  body weight/100). The calculated values are summarized in Table IV. These values are far above actual urine flow measurements reported in the literature (summarized in Table III of Black's review paper (15)). Values reported for salt-water species range from about 3 to 30 ml of urine per kg body weight per day as compared to the average of 118.7 ml/kg/day calculated for salt-water flounder assuming all the radioiodide was excreted renally. Similarly, reported urine flows for fresh-water species range between 7 and 106 ml/kg/day as compared to the 623 ml/kg/day average urine flow calculated for the fresh-water flounder. It is evident that a very large proportion of the excreted  $I^{131}$ , perhaps as much as 80%, is being removed extrarenally in both salt- and fresh-water flounder.

TABLE IV

Calculated urine flows of individual fresh- and salt-water flounder, assuming no extrarenal excretion of  $I^{131}$ . Urinary concentration expressed as % of dose per gram of urine  $\times$  body weight

| Time                 | Urinary concn. | % dose excreted per hr | Calculated urine flow, ml/100 g/hr | ml/kg/day     |
|----------------------|----------------|------------------------|------------------------------------|---------------|
| Salt-water flounder  |                |                        |                                    |               |
| 3:52                 | 3.72           | 3.2                    | 0.9005                             | 225           |
| 4:55                 | 6.05           | 3.25                   | 0.496                              | 119           |
| 6:03                 | 4.99           | 2.43                   | 0.537                              | 129           |
| 7:00                 | 3.42           | 2.1                    | 0.701                              | 168           |
| 7:05                 | 4.91           | 2.15                   | 0.489                              | 117           |
| 7:20                 | 5.92           | 2.0                    | 0.392                              | 94            |
| 18:00                | 2.8            | 0.8                    | 0.282                              | 67.7          |
| 24:25                | 4.42           | 0.35                   | 0.124                              | 29.7          |
|                      |                |                        |                                    | Average 118.7 |
| Fresh-water flounder |                |                        |                                    |               |
| 12:00                | 0.504          | 1.35                   | .90                                | 427           |
| 13:00                | 0.388          | 1.88                   | .85                                | 525           |
| 18:11                | 0.232          | 0.95                   | .66                                | 681           |
| 23:00                | 0.35           | 0.73                   | .57                                | 391           |
| 35:35                | 0.11           | 0.50                   | .50                                | 1090          |
|                      |                |                        |                                    | Average 623   |

The possible pathways of extrarenal iodine removal are the gills, the mucous membranes of the mouth, and with the fecal wastes. In man only minute quantities of iodide are lost extrarenally, that is, in the feces (59, 83), the expired air, and the perspiration (99). In fishes it is also doubtful that appreciable amounts of iodide leave in the feces, particularly in fasted animals such as were used in these experiments. Hence, the gills and mucous membranes must constitute the major organs of extrarenal iodide exchange.

TABLE V

The concentration of  $I^{131}$  in the gill lamellae of starry flounder expressed as percentage of the concentration of  $I^{131}$  in the blood. Body weight 24 to 105 g

| Hours after injection | Fresh-water flounder                    |        | Salt-water flounder<br>(25 ‰ sea water) |        |
|-----------------------|---|--------|---|--------|
|                       | $I^{131}$ concn.<br>in gill lamellae, % | Av., % | $I^{131}$ concn.<br>in gill lamellae, % | Av., % |
| 1                     | 83.6                                    | 83.6   | —                                       | —      |
| 3                     | 40.4, 88.3                              | 64.3   | 109, 267                                | 188    |
| 6                     | 61.7, 69.2                              | 65.4   | 83.4, 108                               | 95.7   |
| 12                    | 147                                     | 147    | 123, 214                                | 168.5  |
| 24                    | 210, 309                                | 259.5  | 79, 157                                 | 118    |
| 48                    | 264, 474                                | 369    | 133, 175                                | 154    |
| 100                   | 208, 212                                | 210    | 128, 175.5, 202                         | 168.5  |

Measurements of the concentration of  $I^{131}$  in the gill lamellae of flounder in both fresh and salt water were undertaken in the hope of providing an indication of the relative importance of the gills in iodide exchange. The results are given in Table V. With the exception of the first 6 hours in the case of fresh-water flounder, the radioiodide concentration is appreciably higher in the gill lamellae than in the blood, indicating a cellular concentration of the isotope. Interpretation, however, is difficult, for it is impossible to say whether the iodide is being concentrated in the gills prior to secretion to the exterior media or whether it represents simply a cellular accumulation with no net movement in either direction. Marine flounder may secrete the univalent iodide ions together with univalent sodium and chloride ions via the "chloride-secreting cells" to the exterior. If so, a concentration of iodide in the gills is not surprising. Fresh-water flounder are not expected normally to secrete iodide, since its concentration is far lower in fresh water than in the blood. There may possibly exist an active iodide-absorption mechanism in the gills of fresh-water flounder (hence the high concentrations of radioiodide in the gill lamellae), but Krogh (68) found that no such mechanism existed in goldfish and that iodide was lost slowly from the body by diffusion.

#### *The Effect of Size*

When a number of starry flounder of all sizes are simultaneously injected with tracer iodide, killed together several hours later, and measurements made of the proportion of the dose excreted, it is seen that small flounder lose the isotope more rapidly than large flounder. An example is shown in

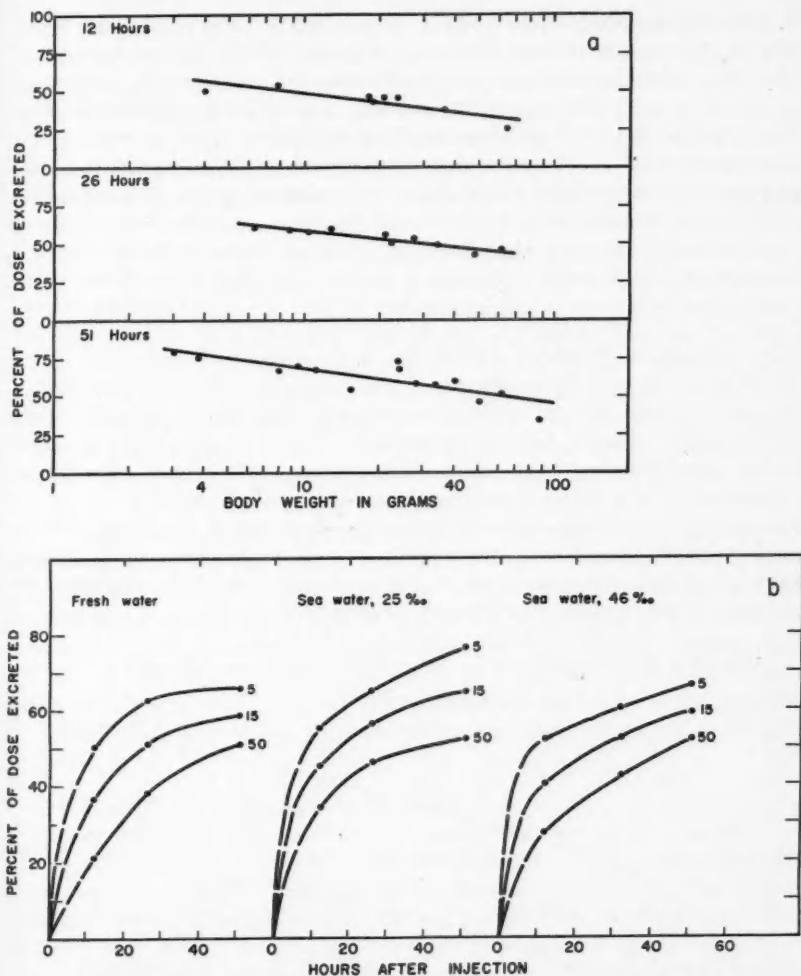


FIG. 18a. Effect of body size on the excretion of radioiodine from *Platichthys stellatus* in sea water of 25‰. Each point represents the proportion of a standard dose of radioiodine excreted by an individual fish at the indicated time after injection. Points fitted by the method of least squares.

FIG. 18b. Effect of body size on the excretion of radioiodine from *Platichthys stellatus* in fresh water, normal sea water, and concentrated sea water. The three points for each curve representing 5-, 15-, and 50-g flounder were graphically derived from lines of best fit through individual total excretion values as shown in Fig. 18a.

Fig. 18a, in which the percentage of dose excreted is plotted against body weight for three sampling periods after injection.

The points best conformed to a linear relationship with body weight placed on a log scale. The results of each of three salinity treatments—46‰,

25‰, and fresh water—were plotted on semilogarithmic paper and fitted with a straight line by the method of least squares. From each of these lines of best fit, three points were graphically derived, representing the total excretion of flounder weighing 5, 15, and 50 g, and plotted against time after injection (Fig. 18*b*). In all three salinities the tracer dose of  $I^{131}$  is more rapidly excreted by small fish. Since the body does not distinguish between stable and radioactive iodide and since the intake of iodide is assumed to balance its loss from the body, it is evident that the net turnover of iodide is greater in small than in large flounder. The relationship between body size and rapidity of iodide exchange is yet another indication of the more intensive metabolic rate of small flounder.

#### *Factors Influencing Uptake of Radioiodide by the Thyroid Gland*

##### *The Rapid Thyroid Accumulation of Radioiodine in Natural Fresh Water*

Figure 19 shows thyroid radioiodine accumulation of six starry flounder, three in natural (iodine-deficient) fresh water (solid circles, solid line) and three in iodine-enriched fresh water (open circles, broken line). The latter was reinforced with 50  $\mu$ g iodine per liter (40  $\mu$ g iodine as  $KIO_3$  and 10  $\mu$ g iodine as KI), the normal iodine content of undiluted sea water (115). Collimated serial in vivo counts were made and corrected for extrathyroidal tissue. Radioiodine-accumulating activity of the thyroid is significantly decreased by the addition of iodine to natural fresh water.

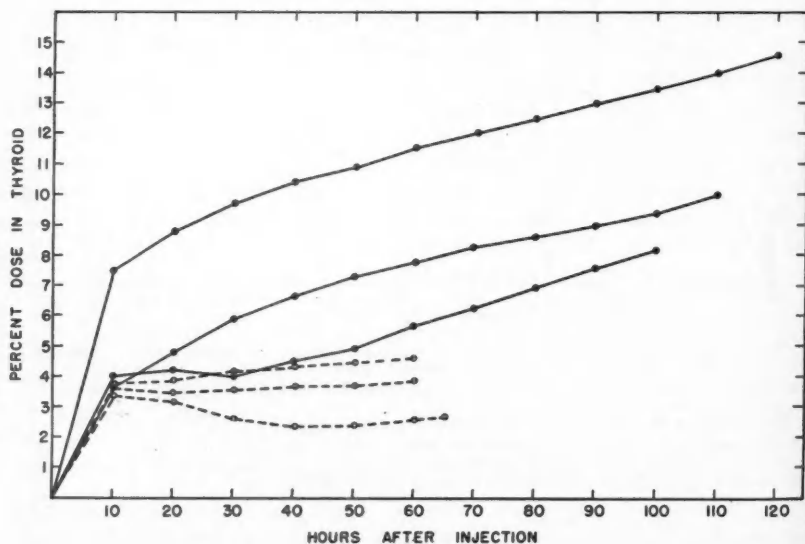


FIG. 19. Effect of iodine content of the water on thyroid radioiodine accumulation of six *Platichthys stellatus*, three in iodine-deficient fresh water (solid circles, solid line) and three in iodine-enriched fresh water (open circles, broken line). Thyroid activity measured in vivo with a collimated scintillation counter.

To assess the effect of low iodine in salt water, artificial sea water was prepared following the formula of Brujewicz (116) using analytical grade reagents. The solution contained the major elements found in sea water (chloride, sodium, magnesium, calcium, potassium, bromide, sulphate, and bicarbonate) without iodine (although iodine undoubtedly is present in very small amounts as a contaminant in the reagents used). Thyroidal uptake of radioiodine by starry flounder was significantly greater in iodine-deficient artificial sea water than in natural sea water of the same salinity (30‰).

The increased rate of thyroidal accumulation of radioiodine by flounder in natural fresh water and artificial sea water as compared to flounder in iodine reinforced fresh water and natural sea water are both interpreted as the familiar compensatory response of the iodide-trapping mechanism to iodine deficiency. As already discussed in the introductory remarks to this section, thyroid hyperplasia as a result of iodine deficiency in the water has been demonstrated in fish by several authors. It is evident that the quantity of iodine available to a fish is an extremely important factor influencing the validity of the radioiodine uptake method as a measure of thyroid activity. Low levels of iodine in the environment stimulate activity of the "iodide trap" resulting in a high rate of radioiodide accumulation that does not represent an over-all increase in release of hormone into the blood stream. This effect is of course particularly important in comparing thyroid activity of flounder in fresh water containing less than 1 µg per liter and in sea water containing about 50 µg per liter (at salinity 35‰). For this reason, fresh water was always reinforced with iodide to bring its iodide concentration equivalent to sea water. Under these conditions, both fresh-water and sea-water flounder were found to have about the same thyroidal radioiodine uptakes.

#### *The Behavior of Radioiodide in the Blood*

Figures 20 and 21 show that several components are present in the blood  $I^{131}$  curves. In both fresh- and salt-water flounder, the concentration in the blood increases rapidly after the intraperitoneal injection as the dose is absorbed into the blood stream. Blood radioiodine of small salt-water flounder (Fig. 20) appears to reach a peak concentration of nearly 5.5% of dose<sup>a</sup> at  $\frac{1}{2}$  hour after injection, followed by a rapid fall to about 2.2% within a few minutes. This rapid rise and fall is interpreted as a rapid absorption of radioiodide into the blood stream via the vascular peritoneum followed by a less rapid diffusion into the tissue spaces.

Frequent serial sampling of blood carried out on large marine flounder immediately following injection showed that absorption of the dose from the coelom is considerably less rapid than in the small flounder. Radioiodide appears not to enter the blood stream more rapidly than it diffuses into the iodide space since its concentration does not reach the high levels seen in

<sup>a</sup>Blood radioiodide concentration is calculated as the percentage of administered dose per gram of blood  $\times$  body weight/100 (biological concentration coefficient), but for brevity in this discussion blood  $I^{131}$  concentration will be expressed simply as percentage of dose.

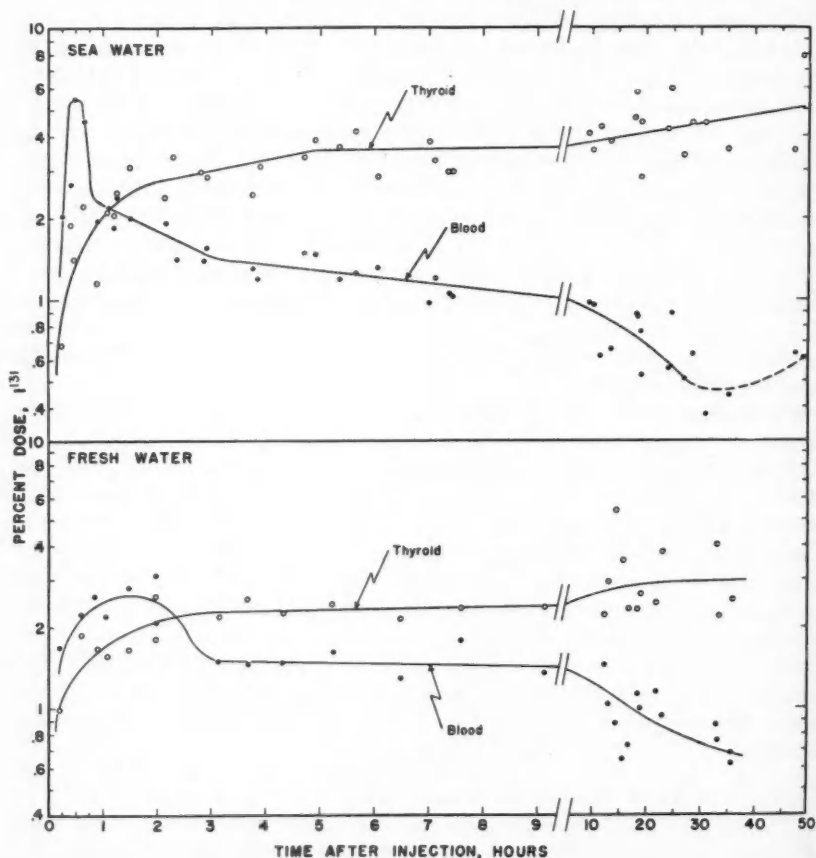


FIG. 20. Effect of salinity on thyroid radioiodine uptake and blood radioiodine disappearance rate of *Platichthys stellatus*. Thyroid uptake (O) is expressed as percentage of dose accumulated by the whole gland; blood radioiodine (●) is expressed as percentage of dose per gram of blood  $\times$  body weight/100. Radioiodine is concentrated more rapidly in the thyroids of sea-water flounder in spite of the more rapid disappearance of radioiodine from the blood. Salinity of sea water = 29‰; fresh water reinforced with 40  $\mu$ g iodine/liter.

small flounder. It will be recalled that disappearance rate of radioiodine from the body of flounder was more rapid in small than in large flounder, indicating a more rapid body turnover of elemental iodine in smaller individuals—another indication of their higher metabolic rate. The absorption of radioiodine from the body cavity of small fresh-water flounder is much slower than in small marine flounder (Fig. 20).

*a. Progressive enlargement of the radioiodide space.*—After absorption, disappearance of radioiodide from the blood of flounder is initially rapid but slows progressively during the first 20–30 hours after injection. An exam-

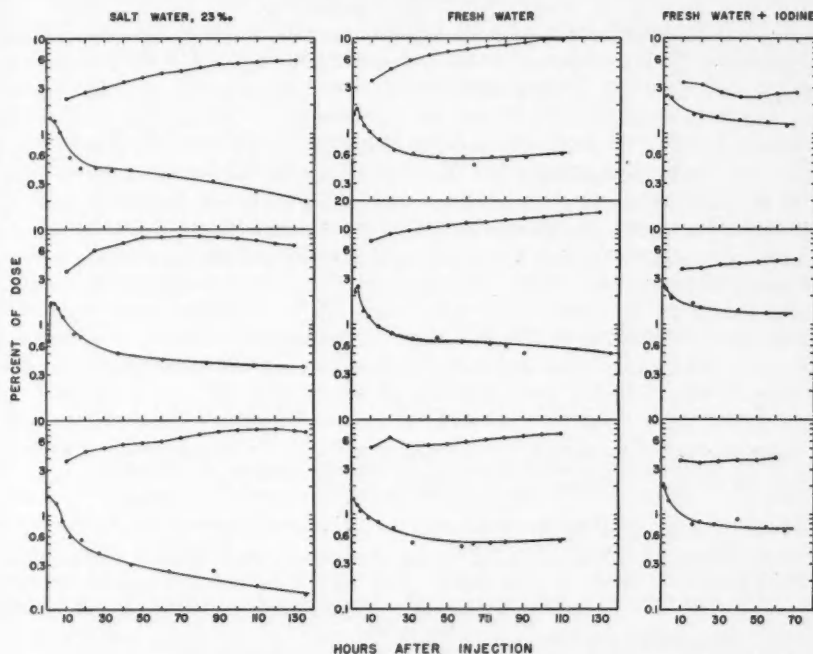


FIG. 21. Effect of salinity on thyroid radioiodine accumulation and blood radioiodine disappearance of nine *Platichthys stellatus*. Thyroid activity (closed circles) was measured in vivo with a collimated scintillation counter. Blood (open circles) was serially sampled from the caudal circulation. Blood activity is expressed as % of dose/gram  $\times$  body weight/100. The graph is discussed in the text.

ination of the blood curves in Fig. 21 shows that in all cases, a semilog plot of blood radioiodide removal during this period does not result in a straight line as would be expected for simple one-phase removal of an isotope. This suggests that the blood radioiodide concentration is decreasing due to a progressive increase in the radioiodide space. An estimate of the radioiodide space, representing the per cent of the total body weight containing radioiodide of the same concentration present in the blood, can be made by dividing the quantity of radioiodide remaining in the entire body (thyroid removed) by the radioiodide concentration in the blood. These calculations have been made for the salt-water flounder in Fig. 20 and plotted in Fig. 22. The radioiodide space is a theoretical rather than a true physical compartment and considerable error may be introduced in the values represented in Fig. 22 by the appearance of organic-bound radioiodine in the blood and by the exclusion of freely diffusable inorganic radioiodine in the thyroid tissue removed. In spite of these deficiencies, there appears to be little doubt that the radioiodide space enlarges with the passage of time. Enlargement is rapid during the first 10–15 hours and apparently continues at a decreasing rate until the dose has diffused essentially throughout the body.

*b. Appearance of organic-bound radioiodine in the blood.*—It has been conclusively demonstrated in mammals that organic-bound hormonal radioiodine appears in the blood a number of hours after injection and circulates in firm combination with certain of the plasma proteins (protein-bound iodine). In this form it remains in the blood for relatively long periods of time. The result is an apparent slowing of radioiodine removal or even a secondary rise in blood activity. While organic radioiodine was not specifically measured in this study, the blood-activity curves strongly suggest that this component does appear in the blood of flounder and significantly influences the shape of these curves. In salt-water flounder, the disappearance of radioiodine from the blood becomes nearly exponential 20 to 30 hours after injection (Fig. 21). Calculation of removal rates for these curves (Table VI) reveals, however, that radioiodine does not disappear as rapidly from the blood as

TABLE VI

Disappearance rate of radioiodine from the blood and nonthyroidal body tissues of starry flounder. Rates calculated between 30th and 70th hours after injection

| Fish No.                    | Disappearance rate from<br>body without thyroid |                             | Disappearance rate<br>from blood |                             |
|-----------------------------|---|-----------------------------|----------------------------------|-----------------------------|
|                             | <i>k</i>  | <i>t</i> $\frac{1}{2}$ , hr | <i>k</i>                         | <i>t</i> $\frac{1}{2}$ , hr |
| Salt water                  |   |                             |                                  |                             |
| 1                           | -.00751   | 92.3                        | -.00491                          | 141.1                       |
| 2                           | -.00914   | 75.8                        | -.00914                          | 75.8                        |
| 3                           | -.00576   | 120.3                       | -.0042                           | 165.0                       |
| 4                           | -.00437   | 158.6                       | -.00131                          | 527.8                       |
| Fresh water                 |   |                             |                                  |                             |
| 5                           | -.00545   | 110.7                       | -.00342                          | 202.6                       |
| 6                           | -.00401   | 172.8                       | -.00248                          | 279.4                       |
| 7                           | -.00491   | 141.0                       | Increase in blood activity       |                             |
| Iodine-enriched fresh water |   |                             |                                  |                             |
| 8                           | -.00482   | 143.7                       | -.00384                          | 180.4                       |
| 9                           | -.00407   | 170.4                       | -.00291                          | 238.1                       |
| 10                          | -.00482   | 143.7                       | -.00468                          | 148.1                       |

from the nonthyroidal tissue of the body. The difference is particularly noticeable in the fresh-water flounder (Fig. 21) where the blood concentration may eventually increase to a higher level. Since inorganic radioiodine continues to be excreted from the bodies of flounder at a steady exponential rate, the most plausible explanation for the significant slowing of the blood radioiodine removal rates in nearly all flounder is that bound radioiodine, presumably hormonal radioiodine, has appeared in the blood. The curves suggest that organic radioiodine appears more rapidly in the blood of flounder in natural fresh water. This is to be expected because the ratio of the quantity of labeled iodine to stable iodine (specific activity) in this iodine-deficient situation is greater than in either sea water or iodine-enriched fresh water. It does not

signify that fresh-water flounder are releasing quantitatively more thyroid hormone from the thyroid gland than are salt-water flounder, but only that a greater proportion of organically bound iodine leaving the gland is tagged. In principle, it should be possible to use the rate of appearance of organic radioiodine as a discriminatory comparison of thyroid activity between salt-water flounder and flounder in iodine-enriched fresh water. However, such would be feasible only if direct measurements of the concentration of labeled hormone in the blood were made. Many variables contribute to the inadequacy of indirect estimates of organic radioiodine secretion based on disappearance rates alone.

*The Effect of Salinity on Thyroid Activity: the Thyroidal Clearance of Radioiodine from the Blood*

Thyroidal clearance of  $I^{131}$  from the blood is determined by simultaneous measurements of thyroid  $I^{131}$  uptake and blood  $I^{131}$  concentration.

$$I^{131} \text{ clearance, thyroid} = \frac{\text{rate of } I^{131} \text{ uptake by the thyroid}}{\text{mean blood concentration of } I^{131}}$$

Because blood  $I^{131}$  concentration is expressed as biological concentration coefficient (% dose/gram  $\times$  body weight/100), clearance is expressed as the volume of blood as per cent body weight cleared of  $I^{131}$ /hour. Both interval sampling and serial in vivo sampling methods were used.

a. *Thyroidal clearance of  $I^{131}$  by small starry flounder.*—Standard tracer doses of  $I^{131}$  were injected into the body cavities of two groups of flounder, one group maintained in sea water and the other previously adapted to iodine-reinforced fresh water. Individual fish were killed at intervals of time after injection, and the thyroid and a blood sample removed from each for counting. The results of one experiment are shown in Fig. 20. Clearance values calculated from these data are given in Table VII. It is apparent that the average thyroid clearance of  $I^{131}$  in fresh-water flounder (about .0135) is much lower than the average clearance calculated for salt-water flounder (about .197).

TABLE VII

Thyroid clearance of radioiodine from the blood of small *Platichthys stellatus*. Clearance rates expressed as the volume of blood (as % body wt) cleared of radioiodine by the thyroid per hour. Rates calculated from data shown in Fig. 20

| Hours after injection | Av. blood $I^{131}$ concn. | Thyroid uptake | Clearance |
|-----------------------|----------------------------|----------------|-----------|
| Fresh-water flounder  |                            |                |           |
| 3-4                   | 1.500                      | .02            | .0133     |
| 4-5                   | 1.485                      | .02            | .0135     |
| 5-6                   | 1.470                      | .02            | .0136     |
| Salt-water flounder   |                            |                |           |
| 2-3                   | 1.6                        | .27            | .169      |
| 3-4                   | 1.38                       | .28            | .202      |
| 4-5                   | 1.32                       | .29            | .22       |

Since the level of elemental iodine in the environment was the same for both groups, these results show conclusively that thyroid iodine trapping activity is greater in salt-water flounder than in fresh-water flounder. The experiment from which these values were derived (Fig. 20) is a replicate of an earlier experiment which also showed much greater clearance rates for salt-water flounder.

*b. Thyroidal clearance of  $I^{131}$  by large starry flounder.*—Thyroidal clearance rates of several larger flounder (average weight about 150 grams) kept in sea water, natural fresh water, and iodine-reinforced fresh water (Fig. 21) are given in Table VIII. Measurements were made by serial in vivo thyroid counting and blood sampling. The values in Table VIII represent the averages of clearance rates calculated over 10-hour intervals between the 10th and 40th hours after injection. Thyroid  $I^{131}$  clearance decreases only slightly with time, the decrease being somewhat more noticeable among the fresh-water flounder. This may support the suggestion already made that organic-bound radioiodine appears more rapidly in the blood of flounder in low iodine fresh water, since

TABLE VIII

Thyroid clearance of radioiodine from the blood of large *Platichthys stellatus*. Clearance rates expressed as the volume of blood (as % body wt.) cleared of radioiodine by the thyroid per hour. Rates calculated from data shown in Fig. 21

| Fish No. | Salt water     | Fish No. | Natural fresh water | Fish No. | Iodine-reinforced fresh water |
|----------|----------------|----------|---------------------|----------|-------------------------------|
| 1        | .0846 ± .0092* | 5        | .0452 ± .0138       | 8        | .00776 ± .00768               |
| 2        | .1352 ± .0396  | 6        | .1213 ± .033        | 9        | .01233 ± .00761               |
| 3        | .0498 ± .0122  | 7        | .1513 ± .0211       | 10       | .0164 ± .00179                |
| 4        | .163 ± .0428   |          |                     | 11       | Nil                           |
| Average  | .0951 ± .0235† |          | .1059 ± .0304       |          | .00912 ± .001739              |

\*Standard deviation.

†Standard error of the mean.

this would tend to elevate blood radioactivity and, hence, decrease thyroid clearance values. However, the appearance of small amounts of organic bound  $I^{131}$  in the blood during the first 40 hours is considered to have a negligible effect on the over-all validity of the clearance calculations. Salt-water and fresh-water flounder showed average thyroid  $I^{131}$  clearance rates of similar magnitude, .095 and .106 per hour respectively. Clearance rates for flounder in iodine-enriched fresh water, however, averaged only about one tenth of those in salt water. These figures are similar to those obtained for small flounder and strengthen the suggestion that thyroid activity is greater in sea water than in fresh water.

#### *Effect of Body Size on Thyroid Activity*

The effect of body size on thyroid activity was assessed by injecting a large number of flounder of variable body size with a standard dose of  $I^{131}$  and sampling groups of fish at predetermined intervals of time after injection.

Thyroid samples were removed, prepared for counting, and their activity measured with the well-crystal scintillation counter, thereby avoiding self-absorption problems due to variable sample mass.

In Fig. 23 an example is shown of the effect of body size on thyroid  $I^{131}$  uptake of flounder in sea water of 25 ‰. The diphasic eye-fitted line placed through the individual thyroïdal uptakes is included only to indicate a trend between uptake and body size and is not to be interpreted as having high significance in itself. Uptakes are relatively high in small flounder of 2 to 6 g, are much less in 20- to 30-g flounder and increase again with increasing body size above 30 g. The same interaction between body size and thyroïdal uptakes was equally prominent in fresh-water-adapted flounder and in flounder adapted to concentrated sea water (45 ‰).

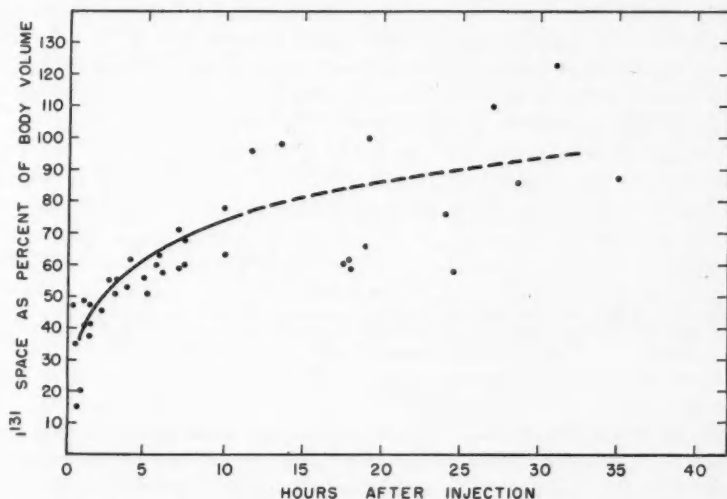


FIG. 22. Enlargement of the radioiodide space (volume of distribution) in marine flounder after intraperitoneal injection of a tracer dose of radioiodine. Each point represents the radioiodine space of an individual fish calculated as:

$$\text{radioiodine space} = \frac{\% \text{ dose } I^{131} \text{ in body without thyroid}}{I^{131} \text{ concentration in the blood}}$$

Since  $I^{131}$  is excreted more rapidly from the body in small than in large flounder (Figs. 22 and 23), the isotope is also disappearing more rapidly from the blood stream. Hence, thyroïdal trapping of  $I^{131}$  by small flounder is proceeding against a lower concentration of  $I^{131}$  in the blood than in large flounder. This means that the actual thyroïdal activity of a 2-g flounder is even greater than indicated by the measured uptakes as compared to the activity of a 20-g flounder. For the same reason, some small part of the measured increase in thyroïdal  $I^{131}$  uptake of larger flounder (100–200 g) may be due to the slower excretion of  $I^{131}$  in these individuals.

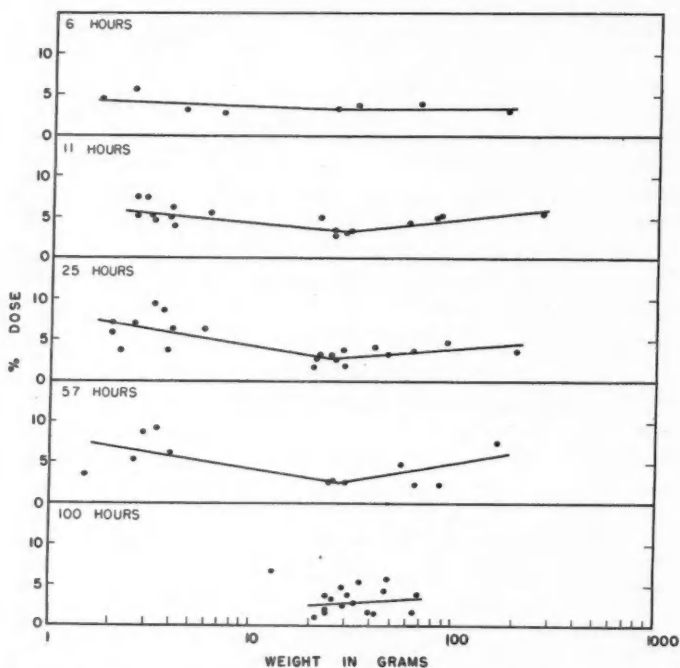


FIG. 23. Effect of body size on thyroid activity of *Platichthys stellatus*. Indicated times represent hours after injection of a tracer dose of radioiodine. Lines fitted by eye.

### Discussion

This investigation demonstrates two facts of significance and suggests an interdependence between them. The first is that energy demands are greater in more saline aquatic environments. The second is that thyroid iodine clearance and presumably thyroid activity is greater in flounder living in marine habitats. If these factors are interrelated it argues strongly for a calorogenic action of thyroid hormone in at least one species of fish—a fact not yet generally recognized. These points will be discussed in turn.

#### *Quantitative Considerations of Energy Demands*

It has been shown that the starry flounder is both a hypotonic and hypertonic regulator, possessing compensating mechanisms for internal osmotic regulation in the face of abrupt and drastic alteration or reversal of osmolarity of the external environment. In this study such alterations were characterized by significant changes in metabolic activity which are assumed equivalent to changed energy needs for osmotic work. An understanding of osmotic energetics necessitates consideration of all processes utilized to maintain internal equilibrium regardless of external osmotic vicissitudes. Starry flounder consumed less oxygen in fresh water than in normal sea water and more

in supernormal salinities. These relationships are shown diagrammatically in Fig. 24. Any proposed explanation must of necessity explain the apparent increasing osmotic work with increasing salt content of the environment. The true explanation lies in relative metabolic demands for the transportation of electrolytes and water in the principal organs of exchange, the gills, the kidney, and the gut. The importance of the integument in limiting permeability to salts and water cannot of course be overemphasized, but low permeability of fish skin is a passive attribute of scales and mucus, rather than an active energy-consuming process. A "differential" permeability may or may not exist but until such a phenomenon is demonstrated it must be assumed that the diffusion of water through the body surface is dependent only on the concentration and direction of the gradient on either side.

While no direct measurements have been made of the oxygen consumption of the perfused teleost kidney or gill doing osmotic work, the recent disclosures of Ussing (117) and Zerahn (123) are of great value in predicting energy demands of these organs for ion transport. In a study of active ion transport by frog skin, these workers found that the amount of oxygen used is more closely related to the amount of sodium transported than to the osmotic work involved. The work is being extended to other biological membranes (117) and the general trend appears to be the same: oxygen consumption bears a constant ratio to the amount of ions transported. The osmotic gradient is of secondary importance.

This new quantitative thermodynamic concept of active transport provides a possible explanation for the lower oxygen consumption of flounder in fresh water than in sea water. Since the salinities used in these experiments represent tonicities of essentially equal though opposite osmotic gradient (fresh water of  $\Delta 0 < \text{body fluid of } \Delta 0.65 < \text{sea water of } \Delta 1.35$ ) it can be assumed that the hydrating and dehydrating effects of fresh and salt water respectively are nearly equal in their disturbing influence on water balance. The difference is found in salt metabolism. In order to maintain osmotic equilibrium

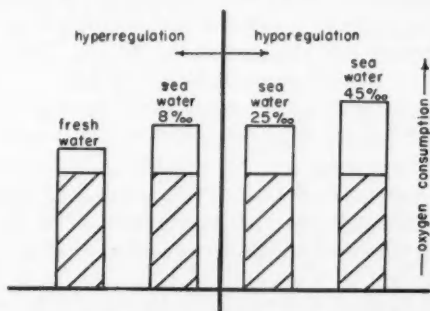


FIG. 24. Diagram of relative energy demands of starry flounder in hypotonic and hypertonic media. Oxygen consumption for "basal" cellular metabolism other than processes connected with osmoregulation are represented by the lower cross-hatched areas of the bars. This portion is assumed to be unaffected by changes in salinity. The upper clear areas represent variable oxygen demands for osmoregulation.

the fresh-water fish excretes osmotic water in a copious urine of low specific gravity. Small amounts of salt are continually lost in the urine and are regained from ingested food and by ion-absorbing cells in the gills and mucous membranes (23, 24, 67, 120). The marine fish, however, has adopted a much more circuitous method for regaining lost osmotic water, that of drinking sea water and excreting the salts via the gills. This method entails more "handling" of salts by the organs of exchange. Salt must be transported across the gastrointestinal mucosa to the blood, carried to the gills, and again secreted across membranes to the external milieu. The process is wasteful of precious body water since the ingested hypertonic sea water must be diluted to isotonicity in the gut (Smith (112) and unreported observations in this laboratory on the starry flounder). While univalent ions (sodium, chloride, and small quantities of potassium) are absorbed from the gut and excreted by the gills, most divalent ions are retained and concentrated in the intestinal residue (114). Some divalents do enter the circulation and are excreted by the kidney, accompanied by an obligatory water loss since the flounder is unable to concentrate urine even to isotonicity. Again active transport is necessary, either to resorb univalent ions from the glomerular filtrate or, what is probably more important in view of the relative unimportance of the latter (31), to secrete selectively divalents into the urine by the tubules from the renal portal circulation. All of this complex ion movement is necessitated by the inability of marine fish to separate specifically water molecules from sea water for direct absorption. It appears, in fact, that no animal cell can pump water into the cell against an osmotic gradient (though water can be pumped out of a cell against an osmotic gradient after it has passively diffused in). Therefore, the distillation of sea water by marine teleosts makes necessary much electrolyte transfer with a concomitant expenditure of energy. In contrast to this, fresh-water fishes have only the relatively simple task of replacing the small amount of salt lost in the urine. Water is free for the fresh-water teleost and this, of course, causes its major osmotic problem, disposing of water without losing salt. It is generally assumed that since fresh-water fish with but one known exception possess glomerular kidneys, the dilute and copious urine is formed from the glomerular filtrate. However, no measurements of either renal blood flows or glomerular filtration rates have been carried out on fresh-water fish. In view of the importance of the renal portal supply to the teleost kidney, it is altogether possible that a greater or lesser portion of the urine represents tubular secretion of water or solutes from this venous supply. The one exception to the apparent universality of glomerular kidneys of fresh-water teleosts, the aglomerular pipefish, *Microphis boaja*, is significant, for it means that tubular secretion alone *can* be adequate for life in fresh water.

Numerous measurements of oxygen consumption by the mammalian kidney have been reported and are summarized by Smith (113). In general, oxygen consumption correlates directly with renal blood flow so that as the latter is decreased so follows the oxygen consumption. Water diuresis has no influence on renal oxygen consumption. These findings would appear to have new

meaning in the light of Ussing's work (117) demonstrating the precise relationship between the amount of sodium transported and the energy (in terms of oxygen consumption) needed for the process. Increased renal blood flow means an increased filtration rate and greater ion reabsorptive activity by the tubules, hence the greater oxygen consumption. Water diuresis would have no effect since the work of filtration is supplied by the heart through the arterial pressure. As long as the filtration rate remains the same, no quantitative alteration in ion reabsorption would occur in spite of the greater urine flow.

Since the rate of glomerular filtration almost certainly increases when the euryhaline flounder moves from salt to fresh water (though studies of this interesting change in renal function are wholly lacking), a concomitant increase in tubular reabsorptive activity for the formation of hypotonic urine would be expected. Whether this process of the fresh-water flounder kidney consumes more energy than the secretion of divalent ions by the kidney of the marine flounder is an open question. Our present lack of knowledge on kidney function in fishes is a prominent obstacle in the way of a clear understanding of teleost osmoregulation. The work of Forster and Berglund (9, 31, 32) has contributed much to an understanding of renal function in marine fishes, but in fresh-water fishes the picture remains obscure.

The results of the standard metabolism experiments show an apparent contradiction, viz.: the oxygen consumption of flounder is less in fresh water than it is in 8‰, a salinity approaching isotonicity. Presumably energy demands should be minimal in the absence of an osmotic gradient to tax energy-consuming homeostatic mechanisms. However, there is no reason to believe that all osmotic mechanisms cease when a euryhaline fish enters isotonic brackish water. It is conceivable that marine starry flounder continue to drink water after entering low salinities, although the process would no longer seem to be efficacious. As long as salt is transported through membranes regardless of the osmotic gradient, energy demands for osmoregulation continue. Again, the lack of experimental evidence precludes further speculation as to the reason for the observed relatively high oxygen consumption in brackish water.

In supernormal salinities (45–50‰) the metabolic rate of starry flounder and speckled sand dab was found to increase considerably above the rate in normal sea water (25‰). As the external salt concentration increases, the osmotic loss of body water through mucous membranes also increases. To restore fluid balance, more sea water must be ingested, and the salt separated and excreted. Since the ingested water is more saline, a much greater quantity of salt must be transported for each gram of water absorbed by the gut. Not only are extrarenal water losses augmented, but it becomes increasingly difficult to replace these losses. In addition, the flounder must excrete more divalent ions via the kidneys and because the urine remains hypotonic even in concentrated sea water (Table I), the obligatory renal water losses are greater. Though no measurements have been made, we would theoretically expect an *increase* in urine flow with a rise in the external osmotic

gradient. A certain relief from this growing problem in water conservation is obtained by allowing the concentration of the body fluids to rise somewhat (Table I). If careful metabolism studies were made over a range of salinities, it would probably be found that the oxygen consumption increases exponentially rather than linearly with an increasing osmotic gradient.

The net result of high salinities is that a greater portion of the animal's basal energy requirements must be devoted to maintaining homoiosmoticity. Fishes are strictly limited in the amount of oxygen available for cellular respiration both by gill limitations and by the low oxygen tensions in the aquatic environment. Hence, any increase in basal metabolic demand is distinctly detrimental to a species, since it decreases the respiratory reserve for activity. Maximal oxygen consumption (active metabolism) is never far above standard metabolic rates, even in active species such as trout or salmon (35, 57). In a high salinity, the "scope for activity" (34) or the difference between active and standard metabolic rates is considerably decreased as a result of augmented energy demands for osmotic work and also because oxygen solubility in water decreases with increasing salt content. Decreases in activity of salmon smolt (*Salmo salar*) moving into salt water from fresh water have been observed (56). In this laboratory, Houston (55) has measured significant decreases in locomotor activity of chum salmon (*Oncorhynchus keta*) moved from fresh to sea water. Although it is uncertain whether decreased activity in sea water is the result of lowered "scope for activity" or is due to a direct inhibitory action on muscle fibers by changes in electrolyte composition of the blood, it is evident that high salinities reduce the physiological reserve of euryhaline fish for other environmental demands.

#### *Action of Thyroid Hormone in Osmoregulation*

Quantitative differences in active ion transport would appear to be the origin of greater demands for thyroid hormone by marine flounder. A possible point of action of thyroid hormone would be a direct effect on the cells doing osmotic work. However, the question of a calorogenic action of thyroid hormone on the cellular metabolism of fishes is by no means clear. The many attempts made to stimulate oxygen consumption of fish with thyroxine or thyroid treatment and the conflicting results obtained are discussed in several reviews (49, 51, 93). Using both fresh water and marine fishes, most authors have reported negative results (2, 27, 28, 46, 52, 94, 101, 110). Chavin and Rossmoore (21) also found thyroxine to have no effect on goldfish respiration, but obtained significant increases in oxygen consumption with thyrotropin treatment. However, Pickford (93) points out that the increase may have been due to contaminating gonadotropin rather than to a direct thyroid stimulation. In spite of these negative reports, Müller (81) showed highly significant increases in oxygen consumption of goldfish after single injections of thyroxine. Haarmann (44) found that an optimum dose of thyroxine stimulated the respiration of isolated muscle of carp.

Studies of the effects of thyroidectomy and thyroid inhibition also have given conflicting results. Neither surgical removal nor radiological destruction

of the thyroids of parrot fish (78) and rainbow trout (33) affected their respiration. The use of thyroid chemical inhibitors has frequently yielded negative results (21, 77) although some authors have reported a depression in respiratory rate with the use of these drugs (81, 90, 122). Hoar (51) has urged that these results be interpreted with caution because of the strong collateral antioxidant effect of some antithyroid materials.

It is questionable whether feeding thyroid or immersion in thyroxine is the most effective way to study the calorogenic action of thyroid hormone. These classical procedures produce all manner of morphogenetic and metabolic effects in fishes (51). However, it is possible that thyroxine is not the active derivative participating in oxidative metabolism. While the iodinated precursors to thyroxine appear to be the same in all vertebrates (7, 39, 40) there is evidence that in mammals thyroxine must be converted to triiodothyronine before it acquires hormonal activity (4, 43). Another criticism of the use of administered thyroid compounds or antithyroid drugs is that selection of optimum dosage is pure guesswork.

In the present study, a correlative decrease in thyroid activity and in metabolic rate has been demonstrated in flounder exposed to a normal physiological situation: a decrease in environmental salinity. This appears to be the first positive demonstration of an apparent calorogenic action of the thyroid hormone in fish not using administered materials such as thyroid hormone or antithyroid compounds. At this point, reference could be made to the work of Olivereau and Francotte-Henry (88), who have suggested that low metabolic rate and slow growth of African blind cavefish (*Caecobarbus geertsi*) may be correlated with the inactive thyroid of this animal.

#### *Influence of Body Size and Gonad Maturation*

The importance of body size in metabolism experiments is frequently overlooked. Reference to Fig. 9 shows that the salinity treatments were inadequate to effect an obvious difference in metabolic rate among large flounder. Only by inclusion of a large size range of flounder did differences attributed to salinity become statistically apparent. Thus, attention to body size is important not only because metabolic rate is weight-dependent but also because changes produced by experimental treatment may show up only among small animals.

As with essentially all animals, metabolic rate of small flounder was greater than large flounder. As a result of their more intense metabolism, smaller flounder excrete a tracer dose of radioiodine considerably faster than do larger individuals (Fig. 18a and 18b). Also disturbances in the osmoconcentration of the body fluids resulting from abrupt salinity alterations were more rapid in smaller flounder (Fig. 2). This is partially due to a more rapid turnover of electrolytes and water by the organs of exchange with the environment and partially due to the proportionately greater surface area of small flounder exposed for osmotic movement of water, and loss or gain of salts. Thyroid activity, on the other hand, forms a striking exception to the proportional decrease in metabolic activity of physiological processes with increasing body

size, for it was shown (Fig. 23) that thyroidal radioiodine uptakes *increased* in flounder above 30 g in weight. These relations are summarized diagrammatically in Fig. 25. The decrease in thyroid activity with increasing body size of small flounder correlates with a concomitant decrease in metabolic rate. The relation may be a morphogenetic one with thyroid activity decreasing with the decreasing rate of growth with increasing body size. This would be in agreement with the theory that the growth-regulating function of the thyroid is more or less independent of the calorigenic function. The effect of thyroid hormone in growth and development is well documented (see reviews by Lynn and Wachowski (75), Hoar (51), and Pickford and Atz (93)) and there is general agreement that thyroid activity is high during periods of metamorphosis. Hoar (49) examined histologically the thyroids of starry flounder in various stages of development. Thyroids appeared active in metamorphosing flounder, while in fully metamorphosed individuals the thyroid had undergone involution. Hoar's findings corresponded with those of Sklower (108) on the European flounder *Pleuronectes platessa*. These histological observations strongly indicate that the decrease in thyroid activity of starry flounder with increasing body size is associated with declining rate of growth. It is not clear to what extent thyroid hormone is involved in growth

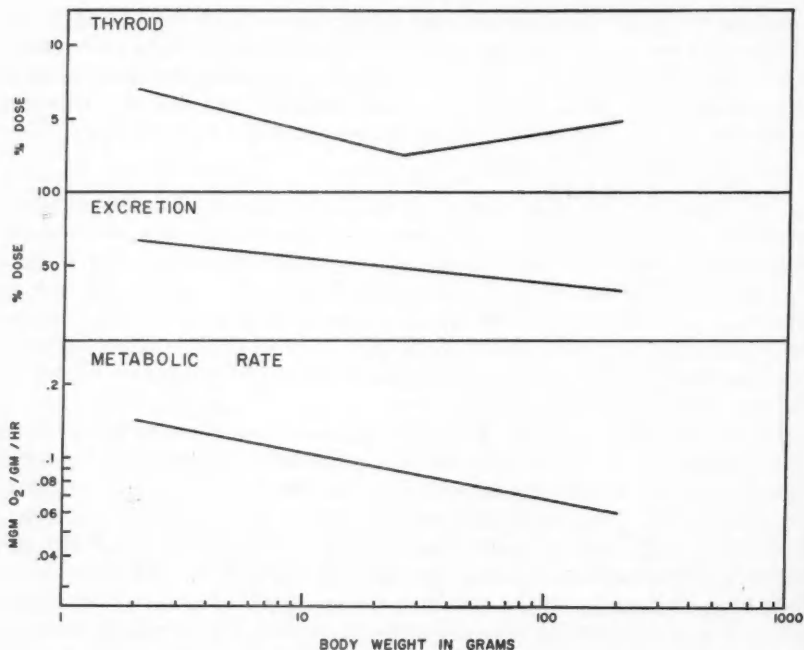


FIG. 25. Diagram of the weight dependency and interrelationships of thyroid activity (% uptake of radioiodine), excretion (% of dose of radioiodine excreted 25 hours after injection), and metabolic rate of starry flounder.

changes, although it is generally felt that it plays a secondary role to the growth hormone but is necessary for the normal expression of the latter.

The systematic increase in thyroid activity in flounder above 30 g appears to be associated with gonad maturation. Several investigators have reported that thyroid hormone is necessary for gonad maturation. Thyroid inhibition retards gonad development (6, 38, 54, 109) while thyroid or thyroxine treatment stimulates the development of secondary sexual characters (36, 54). Increased thyroid activity prior to or during spawning is well known (93). Hoar (49) reported that thyroids of adult starry flounder appeared histologically as active as those of metamorphosing flounder. These findings and others are suggestive of some correlate between thyroid activity and the sexual cycle although its significance is presently obscure.

#### *Hormone Specificity*

The point of particular interest to be noted from Fig. 25 is that the increase in thyroid activity in flounder above 30 g is without effect on total metabolic rate. If the increase in thyroid radioiodine uptake is truly indicative of greater hormone secretion into the blood stream, it is significant that oxygen consumption is not stimulated. This suggests that the thyroid hormone may have rather specific effects on cells of the body. The increased thyroid activity in sea water is also suggestive of hormone specificity, in this case a direct effect on oxidative metabolism of cells doing osmotic work.

The work of Barker (4, 5) indicates that mammalian tissues respond very selectively to thyroid hormone with respect to metabolic rate; liver, kidney, gastric mucosa, salivary glands, pancreas, and various muscle tissues responded to thyroid hyper- or hypo-activity, whereas brain, spleen, thymus, and various reproductive organs did not respond. Unfortunately, few studies have been carried out on the effect of thyroid hormone on the metabolism of tissues of cold-blooded vertebrates. In the present studies the inflection of the thyroid activity curve in 20- to 30-g flounder suggests some direct or indirect involvement between thyroid hormone and gonadal development with no effect on the oxidative metabolism of these tissues. If subsequent research on the thyroid-reproductive relationship in fishes supports this explanation, the results, together with the suggestion of a specific effect on energy metabolism of osmoregulatory tissues, appear to support the thesis that in lower vertebrates, the developmental and calorogenic actions of thyroid hormone are two independent effects.

In this paper, experimental evidence has been presented demonstrating a consistent interrelationship between energy demands for osmoregulation and thyroid activity of the euryhaline starry flounder. Both metabolic rate and the activity of the thyroid gland are significantly less in fresh water than in sea water. The decrease in metabolic rate is not inconsistent with theoretical energy demands for osmoregulation by fish. Because marine fish rely upon a more circuitous osmoregulatory mechanism than fresh-water fish, it is expected that active ion transport is quantitatively greater in the former. If, as recent evidence suggests, oxygen consumption is more related to the

amount of ions transported than to the osmotic gradient present, the greater energy demands of marine flounder is explained on this basis.

When the mild goitrogenic effect of natural fresh water is removed by addition of iodine in amounts equivalent to that found in sea water, thyroidal radioiodine clearance rates were much greater in the marine habitat. This correlate between thyroid activity and metabolic rate strongly implicates a positive calorogenic action of the thyroid hormone.

Many questions remain unanswered. It is not known, for instance, where or in what way thyroid hormone exercises its influence in osmoregulation, other than it presumably stimulates oxidative metabolism of the cells doing osmotic work. As in higher vertebrates, the thyroid hormone of fish may act rather specifically on certain cells and tissues of the body. Not all of this action is calorogenic. Reference was made to the increased thyroid activity of flounder during gonad maturation with no significant effect on total metabolic rate. Yet, the latter notwithstanding, the many fruitless attempts to show a calorogenic action of the fish thyroid hormone should by no means be regarded as conclusive evidence that such an action is not present. Perhaps the real significance of the thyroid in fish lies in its ability to aid adaptation of the organism to environmental vicissitudes, such as osmotic and temperature stress (53), and to periods of rapid internal change such as metamorphosis and sexual maturation.

### Acknowledgments

It is a pleasure to acknowledge my indebtedness and gratitude to Dr. W. S. Hoar for his invaluable advice and encouragement throughout this study. Also gratefully acknowledged is the generous assistance of Drs. P. A. Dehnell, P. A. Larkin, E. C. Black, and Mr. R. R. Parker. I am obligated to Dr. B. Baggerman and Dr. A. H. Houston for helpful advice and many stimulating discussions during the course of the study.

The use of the end-probe scintillation counter was generously supplied by Dr. D. H. Copp, Department of Physiology. Dr. P. F. Solvonuk, British Columbia Medical Research Institute, kindly granted the use of the well-crystal scintillation counter. A continual supply of live flatfish was made available through the kind assistance of Mr. Joseph Bauer of Steveston, British Columbia, and Mr. Norman Pelkey of Vancouver, British Columbia.

The Vancouver Public Aquarium Association kindly provided many research facilities.

Financial support for this research was received from the National Research Council of Canada through grants-in-aid of research to W. S. Hoar and a studentship to the author.

### References

1. BAGGERMAN, B. An experimental study on the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.). Arch. néerl. zool. **12**(2), 105-317 (1957).
2. BARADUC, M. M. Influence de la thyroxination de jeunes truites arc-en-ciel (*Salmo gairdneri*) sur la teneur en lipides viscéraux et periviscéraux. Compt. rend. **238**, 728-730 (1954).

3. BARKER, S. B. Mechanism of action of the thyroid hormone. *Physiol. Revs.* **31**, 205-243 (1951).
4. BARKER, S. B. The circulating thyroid hormone. *Brookhaven Symp. Biol.* **7**, 74-88 (1955).
5. BARKER, S. B. and SCHWARTZ, H. S. Further studies on metabolism of tissues from thyroxin-injected rats. *Proc. Soc. Exptl. Biol. Med.* **83**, 500-502 (1953).
6. BARRINGTON, E. J. W. and MATTY, A. J. Influence of thiourea on reproduction in the minnow. *Nature*, **170**, 105-106 (1952).
7. BERG, O. and GORBMAN, A. Utilization of iodine by the thyroid of the platyfish, *Xiphophorus* (*Platyopocilus*) *maculatus*. *Proc. Soc. Exptl. Biol. Med.* **83**, 751-756 (1953).
8. BERG, O. and GORBMAN, A. Normal and altered thyroidal function in domesticated goldfish, *Carassius auratus*. *Proc. Soc. Exptl. Biol. Med.* **86**, 156-159 (1954).
9. BERGLUND, F. and FORSTER, R. P. Renal tubular transport of inorganic divalent ions by the glomerular marine teleost, *Lophius americanus*. *J. Gen. Physiol.* **41**, 429-440 (1958).
10. BERKSON, J., KEATING, F. R., JR., POWER, M. H. and MCCONAHEY, W. M. Determination of renal clearance of radioiodine. *J. Appl. Physiol.* **2**, 522-529 (1950).
11. BERSON, S. A., YALOW, R. S., SORRENTINO, J., and ROSWIT, B. The determination of thyroidal and renal plasma  $I^{131}$  clearance rates as a routine diagnostic test of thyroid dysfunction. *J. Clin. Invest.* **31**, 141-158 (1952).
12. BERTALANFFY, L. von. Metabolic rate and growth types. *Am. Naturalist*, **85**, 111-117 (1951).
13. BERTALANFFY, L. von. Quantitative laws in metabolism and growth. *Quart. Rev. Biol.* **32**, 217-231 (1957).
14. BLACK, E. C., FRY, F. E. J., and SCOTT, W. J. Maximum rates of oxygen transport for certain freshwater fish. *Anat. Record*, **75**, Suppl. 103, 80 (1939).
15. BLACK, V. S. Excretion and osmoregulation, in *The physiology of fishes*. Edited by M. E. Brown, Vol. 1. Academic Press Inc., New York. 1957. Chap. 4, pp. 163-205.
16. BRUCK, E. K. and GOLDBERG, I. J. L. The use of simple thyroid function tests in the diagnosis of hyperthyroidism. *J. Endocrinol.* **16**, 277-282 (1958).
17. BURDEN, C. E. The failure of hypophysectomized *Fundulus heteroclitus* to survive in fresh water. *Biol. Bull.* **110**, 8-28 (1956).
18. BUSHNELL, R. G., DRILHON, A., and RAFFY, A. Recherches sur la physiologie des Salmonides. *Bull. inst. océanog. (Monaco)*. No. 893 (1946).
19. CHAVIN, W. Thyroid follicles in the head kidney of the goldfish, *Carassius auratus* (Linnaeus). *Zoologica*, **41**(3), 101-104 (1956).
20. CHAVIN, W. Thyroid distribution and function in the goldfish, *Carassius auratus* L. *J. Exptl. Zool.* **133**, 259-279 (1956).
21. CHAVIN, W. and ROSSMOORE, H. W. Pituitary-thyroid regulation of respiration in the goldfish, *Carassius auratus* L. *Anat. Record*, **125**, 599 (1956).
22. COMAR, C. L. Radioisotopes in biology and agriculture. McGraw-Hill Book Co. Ltd., Toronto. 1955.
23. COPELAND, D. E. The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. *J. Morphol.* **82**, 201-227 (1948).
24. COPELAND, D. E. The adaptive behaviour of the chloride cell in the gill of *Fundulus heteroclitus*. *J. Morphol.* **87**, 369-380 (1950).
25. CORDIER, D. and LEBLANC, M. Influence du passage de l'eau de mer à l'eau douce sur les échanges respiratoires et l'absorption intestinale du glucose chez la rascasse (*Scorpaena porcus*, L.). *J. physiol. (Paris)*, **47**, 719-724 (1955).
26. CORDIER, D. and MAURICE, A. Influence du passage de l'eau douce à l'eau salée sur les échanges respiratoires et l'absorption intestinale du glucose chez la tanche (*Tinca vulgaris*, L.). *Acta Physiol. et Pharmacol. Neerl.* **6**, 431-439 (1957).
27. DREXLER, E. and ISSEKUTZ, B. V. Die Wirkung des Thyroxins auf den stoffwechsel kaltblütiger Wirbeltiere. *Arch. exptl. Pathol. Pharmacol.* **177**, 435-441 (1935).
28. ETKIN, W. N., ROOT, R. W., and MORSHIN, B. P. The effect of thyroid feeding on oxygen consumption of goldfish. *Physiol. Zool.* **13**, 415-429 (1940).
29. FONTAINE, M. Équilibre hydrominéral et quelques particularités de sa régulation chez les vertébrés. *Arch. Sci. Physiol.* **7**, C55-77 (1953).
30. FONTAINE, M. The hormonal control of water and salt-electrolyte metabolism in fish. *Mem. Soc. Endocrinol.* **5**, 69-81 (1956).
31. FORSTER, R. P. A comparative study of renal function in marine teleosts. *J. Cellular Comp. Physiol.* **42**, 487-510 (1953).
32. FORSTER, R. P. and BERGLUND, F. Osmotic diuresis and its effect on total electrolyte distribution in plasma and urine of the glomerular teleost, *Lophius americanus*. *J. Gen. Physiol.* **39**, 349-359 (1956).
33. FROMM, P. O. and REINEKE, E. P. Some aspects of thyroid physiology in rainbow trout. *J. Cellular Comp. Physiol.* **48**, 393-404 (1956).

34. FRY, F. E. J. Effects of the environment on animal activity. Univ. Toronto Stud. Biol. Ser. No. 55; Publ. Ont. Fish. Research Lab. No. 68 (1947).
35. FRY, F. E. J. The aquatic respiration of fish, in *The physiology of fishes. Edited by M. E. Brown. Vol. 1. Academic Press Inc., New York. 1957. Chap. 1, pp. 1-63.*
36. GAISER, M. L. Effets produits par l'administration prolongée de thiourée et de thyroxine chez *Lebistes reticulatus*. Compt. rend. soc. biol. **146**, 496-498 (1952).
37. GEYER, F. and MANN, H. Die Bedeutung der Grösse der Atemkammer für den Sauerstoffverbrauch in fließendem Wasser. Z. vergleich. Physiol. **27**, 443-444 (1939).
38. GOLDSMITH, E. D., NIGRELLI, R. F., GORDON, A. S., CHARIPPER, H. A., and GORDON, M. Effect of thiourea upon fish development. Endocrinology, **35**, 132-133 (1943).
39. GORBMAN, A. and BERG, O. Thyroid function in the fishes *Fundulus heteroclitus*, *F. majalis*, and *F. diaphanus*. Endocrinology, **56**, 86-92 (1955).
40. GORBMAN, A., LISSITZKY, S., MICHEL R., and ROCHE, J. Thyroid metabolism of iodine in the shark *Scyliorhinus (Scyllium) canicula*. Endocrinology, **51**, 311-321 (1952).
41. GRAETZ, E. Versuch einer exakten Analyse der zur Osmoregulation benötigten Kräfte in ihrer Beziehung zum Gesamtstoffwechsel von Süßwasserstichlingen in hypo- und hypertonen Medien. Zool. Jahrb. Abt. Allgem. Zool. Physiol. **49**, 37-58 (1931).
42. GROSS, J. Osmotic responses in the sipunculid *Dendrostomum zosteriolum*. J. Exptl. Biol. **31**, 402-423 (1954).
43. GROSS, J. The distribution of radioactive thyroid hormone in tissues. Brookhaven Symp. Biol. **7**, 102-109 (1955).
44. HAARMANN, W. Über den Einfluss von Thyroxin auf den Sauerstoffverbrauch über lebender Gewebe. Arch. Exptl. Pathol. Pharmacol. **180**, 167-182 (1936).
45. HAMRE, C. and NICHOLS, M. S. Exophthalmia in trout-fry. Proc. Soc. Exptl. Biol. Med. **26**, 63-65 (1926).
46. HASLER, A. D. and MEYER, R. K. Respiratory responses of normal and castrated goldfish to teleost and mammalian hormones. J. Exptl. Zool. **91**, 391-404 (1941).
47. HENSCHEL, J. Wasseraushalt und Osmoregulation von Scholle und Flunder. Wiss. Meeresunt. Abt. Kiel. N. F. **22**, 89-121 (1936).
48. HEUTS, M. J. La régulation osmotique chez l'épinochette (*Pygosteus pungitius* L.). Ann. Soc. Zool. Belg. **74**, 99-105 (1943).
49. HOAR, W. S. Hormones in fish. Univ. Toronto Biol. Ser. No. 59, Publ. Ont. Fisheries Research Lab. No. 71, 1-51 (1951).
50. HOAR, W. S. Thyroid function in some anadromous and landlocked teleosts. Trans. Roy. Soc. Canada V, **46**, 39-53 (1952).
51. HOAR, W. S. Endocrine organs, in *The physiology of fishes. Edited by M. E. Brown, Academic Press Inc., New York. 1957. Chap. 6, pp. 245-285.*
52. HOAR, W. S. Effects of synthetic thyroxine and gonadal steroids on the metabolism of goldfish. Can. J. Zool. **36**, 113-121 (1958).
53. HOAR, W. S. Endocrine factors in the ecological adaptation of fishes. In *Comparative Endocrinology. Edited by A. Gorbman. John Wiley & Sons, New York. 1959.*
54. HOPPER, A. F. Growth and maturation response of *Lebistes reticulatus* to treatment with mammalian thyroid powder and thiouracil. J. Exptl. Zool. **119**, 205-217 (1952).
55. HOUSTON, A. H. Locomotor performance and osmoregulation in juvenile anadromous salmonids following abrupt environmental salinity change. Doctoral dissertation, Univ. British Columbia (1958).
56. HUNTSMAN, A. G. and HOAR, W. S. Resistance of Atlantic salmon to sea water. J. Fisheries Research Board Can. **4**, 409-411 (1939).
57. JOB, S. V. The oxygen consumption of *Salvelinus fontinalis*. Univ. Toronto Biol. Ser. No. 61, Publ. Ont. Fisheries Research Lab. No. 73 (1955).
58. JØRGENSEN, C. B. and ROSENKILDE, P. On regulation of concentration and content of chloride in goldfish. Biol. Bull. **110**, 300-305 (1956).
59. KEATING, F. R., JR., and ALBERT, A. The metabolism of iodine in man as disclosed with the use of radioiodine. Recent Progr. Hormone Research, **4**, 429-481 (1948).
60. KEATING, F. R., JR., HAINES, S. F., POWER, M. H., and WILLIAMS, M. M. D. The radioiodine-accumulating function of the human thyroid gland as a diagnostic test in clinical medicine. J. Clin. Endocrinol. **10**, 1425-1464 (1950).
61. KETCHEN, K. S. Studies on lemon sole development and egg production. Fisheries Research Board Can. Pac. Progr. Rept. No. 73 (1947). pp. 68-70.
62. KETCHEN, K. S. Factors influencing the survival of the lemon sole (*Parophrys vetulus*) in Hecate Strait, British Columbia. J. Fisheries Research Board Can. **13**, 647-694 (1956).
63. KEYS, A. B. The measurement of the respiratory exchange of aquatic animals. Biol. Bull. **59**, 187-198 (1930).
64. KEYS, A. B. Chloride and water secretion and absorption by the gills of the eel. Z. vergleich. Physiol. **15**, 364-388 (1931).

65. KOCH, H. J. and HEUTS, M. J. Régulation osmotique, cycle sexuel et migration de reproduction chez les épinosches. Arch. intern. physiol. **53**, 253-266 (1943).
66. KROGH, A. The quantitative relation between temperature and standard metabolism in animals. Intern. Z. physik. chem. Biol. **1**, 491-508 (1914).
67. KROGH, A. Osmotic regulation in fresh water fishes by active absorption of chloride ions. Z. vergleich. Physiol. **15**, 364-388 (1937).
68. KROGH, A. Osmotic regulation in aquatic animals. The University Press, Cambridge. 1939.
69. KUENEN, D. J. Systematical and physiological notes on the brine shrimp, *Artemia*. Arch. néerl. Zool. **3**, 365-449 (1939).
70. LA ROCHE, G. Fixation du radioiode par la glande thyroïde du saumon de l'Atlantique. Ann. ACFAS, **16**, 134-137 (1950).
71. LA ROCHE, G. Effets de préparations thyroïdiennes et d'iodures sur le goitre (pseudo-cancer) des salmonidés. Rev. can. biol. **11**, 439-445 (1953).
72. LEINER, M. Die Physiologie der Fischatmung. Leipzig. 1938.
73. LELOUP, J. Influence d'un abaissement de salinité sur la cuprémie de deux téléostéens marins: *Muraena helena* L., *Labrus bergylla* Asc. Compt. rend. soc. biol. **142**, 178-179 (1948).
74. LINDROTH, A. Sauerstoffverbrauch der Fische. II. Verschiedene Entwicklungs- und Alterstadien vom Lachs und Hecht. Z. vergleich. Physiol. **29**, 583-594 (1942).
75. LYNN, W. G. and WACHOWSKI, H. E. The thyroid gland and its functions in cold-blooded vertebrates. Quart. Rev. Biol. **26**, 123-168 (1951).
76. MARINE, D. J. and LENHART, C. H. Observations and experiments on the so-called thyroid carcinoma of brook trout (*Salvelinus fontinalis*) and its relation to ordinary goitre. J. Exptl. Med. **12**, 311-337 (1910).
77. MATTHEWS, S. A. and SMITH, D. C. The effect of thiourea on the oxygen consumption of *Fundulus*. Physiol. Zool. **20**, 161-164 (1947).
78. MATTY, A. J. Thyroidectomy and its effect upon oxygen consumption of a teleost fish, *Pseudoscarias guacamaia*. J. Endocrinol. **15**, 1-8 (1957).
79. MCCONAHEY, W. M., KEATING, F. R., JR., POWER, M. H. An estimation of the renal and extrarenal clearance of radioiodide in man. J. Clin. Invest. **30**, 778 (1951).
80. MEYER, D. K., WESTFALL, B. A., and PLATNER, W. S. Water and electrolyte balance of goldfish under conditions of anoxia, cold and inanition. Am. J. Physiol. **184**, 553-556 (1956).
81. MÜLLER, J. Über die Wirkung von Thyroxin und Thyreotropem Hormon auf den Stoffwechsel und die Färbung der Goldfische. Z. vergleich. Physiol. **35**, 1-12 (1953).
82. MYANT, N. B., POCHIN, E. E., and GOLDIE, E. A. G. The plasma iodide clearance rate of the human thyroid. Clin. Sci. **8**, 109-131 (1949).
83. NELSON, N., PALMES, E. D., PARK, C. R., WEYMOUTH, P. P., and BEAN, W. B. The absorption, excretion, and physiological effect of iodine in normal human subjects. J. Clin. Invest. **26**, 301-310 (1947).
84. OHLE, W. The chemical and electrochemical determination of the dissolved molecular oxygen in fresh waters. Intern. Assoc. Theoretical and Appl. Limnology, Commun. No. 3, 1-44 (1953). (In German.)
85. OLIVEREAU, M. Influence d'une diminution de salinité sur l'activité de la glande thyroïde de deux téléostéens marins: *Muraena helena* L. et *Labrus bergylla* Asc. Compt. rend. soc. biol. **142**, 176-177 (1948).
86. OLIVEREAU, M. Influence d'une augmentation de salinité sur l'activité thyroïdienne des diverses téléostéens d'eau douce. Compt. rend. soc. biol. **144**, 775-776 (1950).
87. OLIVEREAU, M. Hypophyse et glande thyroïde chez les poissons. Étude histophysiologique de quelques corrélations endocriniennes, en particulier chez *Salmo salar* L. Ann. Inst. océanogr. Monaco, **29**, 95-296 (1954).
88. OLIVEREAU, M. and FRANCOIS-HENRY, M. Étude histologique et biométrique de la glande thyroïde de *Caecobarbus geertsii*. Ann. Soc. Zool. Belg. **86**, 129-150 (1956).
89. ORCUTT, H. G. The life history of the starry flounder, *Platichthys stellatus* (Pallas). Calif. Div. Fish and Game, Fisheries Bull. **78** (1950).
90. OSBORN, P. E. Some experiments on the use of thiouracil as an aid in holding and transporting fish. Progr. Fish-Cult. **13**, 75-79 (1951).
91. OSTLE, B. Statistics in research. The Iowa State College Press, Ames, Iowa. 1954.
92. PAULIK, G. J. and DELACY, A. C. Changes in the swimming ability of Columbia River sockeye salmon during upstream migration. Univ. Wash. College Fisheries Tech. Rept. No. 46. (1958).
93. PICKFORD, G. E. and ATZ, J. W. The physiology of the pituitary gland of fishes. New York Zoological Society, New York. 1957.
94. PUNT, A. and JONGBLOED, J. On factors influencing the gas exchanges in fish. Arch. néerl. Zool. **7**, 1-15 (1945).
95. RAFFY, A. Recherches physiologiques sur le mécanisme de la mort des poissons sténohalins soumis à des variations de salinité. Bull. inst. océanogr. (Monaco), No. 602 (1932).

96. RAFFY, A. Recherches sur le métabolisme respiratoire des poikilothermes aquatiques. *Ann. inst. océanog.* (Monaco), **13**, 259-393 (1933).
97. RAFFY, A. L'euryhalinité des jeunes plies *Pleuronectes platessa* L. *Compt. rend. soc. biol.* **149**, 2122-2123 (1955).
98. RAFFY, A. and FONTAINE, M. De l'influence des variations de salinité sur la respiration des civelles. *Compt. rend. soc. biol.* **104**, 466-468 (1930).
99. RIGGS, D. S. Quantitative aspects of iodine metabolism in man. *Pharm. Rev.* **4**, 284-370 (1952).
100. ROBERTSON, O. H. and CHANEY, A. L. Thyroid hyperplasia and tissue iodine content in spawning rainbow trout: a comparative study of Lake Michigan and California sea-run trout. *Physiol. Zool.* **26**, 328-340 (1953).
101. ROOT, R. W. and ETKIN, W. Effect of thyroxine on the oxygen consumption of the toadfish. *Proc. Soc. Exptl. Biol. Med.* **37**, 174-175 (1937).
102. SCHLIEPER, C. Ueber die Einwirkung nieder Salzkonzentration auf marine Organismen. *Z. vergl. Physiol.* **9**, 478-514 (1929).
103. SCHLIEPER, C. Neuere Ergebnisse und Probleme aus dem Gebiet der Osmoregulation wasserlebender Tiere. *Biol. Revs.* **10**, 334-360 (1935).
104. SCHLUMBERGER, H. G. Spontaneous hyperplasia and neoplasia in the thyroid of animals. *Brookhaven Symp. Biol. No. 7*, 169-191 (1955).
105. SCHULTZ, A. L. and ZIEVE, L. Thyroid clearance, uptake, and rate of uptake of radioiodine in hyperthyroidism. *J. Lab. Clin. Med.* **50**(3), 335-347 (1957).
106. SCHULTZ, A. L. and ZIEVE, L. Relationships between clinical severity of hyperthyroidism and results of thyroid function tests. *J. Clin. Endocrinol. and Metabolism*, **18**, 629-635 (1958).
107. SHAMARDINA, I. P. Alteration of the intensity of fish respiration during development. *Akad. Nauk S.S.S.R., Doklady*, **98**, 669-672 (1954). (In Russian.)
108. SKLOWER, A. Untersuchungen über die inkretorischen Organe der Fische. II. Die Metamorphose der Plattfische und die Bedeutung der Schilddrüse für diesen Vorgang. *J. Conseil*, **14**, 81-85 (1939).
109. SMITH, D. C., SLADEK, S. A., and KELLNER, A. W. The effect of mammalian thyroid extract on the growth rate and sexual differentiation in the fish, *Lebistes reticulatus*, treated with thiourea. *Physiol. Zool.* **26**, 117-124 (1953).
110. SMITH, D. C. and EVERETT, G. M. The effect of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish, *Lebistes reticulatus*. *J. Exptl. Zool.* **94**, 229-240 (1943).
111. SMITH, D. C. W. The role of the endocrine organs in the salinity tolerance of the trout. *Mem. Soc. Endocrinol.* **5**, 83-98 (1956).
112. SMITH, H. W. The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.* **93**, 480-505 (1930).
113. SMITH, H. W. The kidney. Structure and function in health and disease. Oxford Univ. Press, New York, 1951.
114. SMITH, H. W. From fish to philosopher. Little, Brown and Co., Boston, 1953.
115. STRICKLAND, J. D. H. Personal communication. Pacific Biological Station, Nanaimo, B. C. 1956.
116. SVERDRUP, H. U., JOHNSON, M. W., and FLEMING, R. H. The oceans. Prentice-Hall, Inc., New York, 1942.
117. USSING, H. H. Personal communication. Zoophysiological Laboratory, Copenhagen, 1958.
118. VESELOV, E. A. Effect of salinity of the environment on the rate of respiration in fish. *Zool. Zhurnal*, **28**, 85-98 (1949). (In Russian.)
119. WELLS, N. A. The importance of the time element in the determination of the respiratory metabolism in fishes. *Proc. Natl. Acad. Sci. (U.S.)*, **18**, 580-585 (1932).
120. WIKGREN, B. Osmotic regulation in some aquatic animals with special reference to the influence of temperature. *Acta Zool. Fennica*, **71**, 1-102 (1953).
121. WOHLISCHLAG, D. E. Differences in metabolic rates of migratory and resident freshwater forms of an Arctic whitefish. *Ecology*, **38**, 502-510 (1957).
122. ZAKS, M. G. and ZAMKOVA, M. A. On the influence of thiourea on the gaseous exchanges of the larvae of salmon and seviuga. *Doklady Akad. Nauk USSR*, **84**, 1101-1103 (1952). (In Russian.)
123. ZERAHN, K. Oxygen consumption and active sodium transport in the isolated and short-circuited frog skin. *Acta Physiol. Scand.* **36**, 300-318 (1956).
124. ZEUTHEN, E. Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. *Compt. rend. Lab. Carlsberg, Sér. chim.* **26**, 17-161 (1947).
125. ZEUTHEN, E. Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.* **28**, 1-12 (1953).
126. ZEUTHEN, E. Comparative physiology (respiration). *Ann. Rev. Physiol.* **17**, 459-482 (1955).

## EFFECT OF OXYGEN DEFICIENCY ON RADIATION-INDUCED MITOTIC DAMAGE IN SYNCHRONOUSLY DIVIDING CELLS<sup>1</sup>

W. F. BALDWIN AND T. N. SALTHOUSE

### Abstract

The latent effects of X irradiation in delaying mitosis are readily observable in the epidermis of the insect *Rhodnius* owing to the degree of synchrony of division in these cells following a blood meal. At the dose employed in these studies, mitosis did not proceed beyond metaphase when the insects were exposed in air; after irradiation at the same dose in nitrogen, a prolonged division was completed with the greater part of the inhibition occurring during metaphase.

Increased radioresistance associated with a lowering of the oxygen tension during exposure has been studied in a number of living systems, including yeast, broad bean root tips, *Drosophila*, mouse tumor cells, and in whole mammals (1). In the insect *Rhodnius*, molting times and the sizes of latent radiation burns in the cuticle were both reduced by a factor of two to three when the insects were exposed in an atmosphere of nitrogen instead of air (2). The onset of cell division in *Rhodnius* epidermis prior to the formation of a new cuticle is essentially synchronous, and mitosis occurs only after a fixed period following a meal of blood (3). Thus the effects of radiation during a resting stage on the behavior of the cells can be studied at a subsequent division more readily than in tissues having asynchronous division. In the present study, radiation was observed to delay both the completion of mitosis in epidermal cells and the subsequent molting process; further, the protection given by oxygen deficiency during exposure was associated with the ability of irradiated cells to pass through a prolonged metaphase stage.

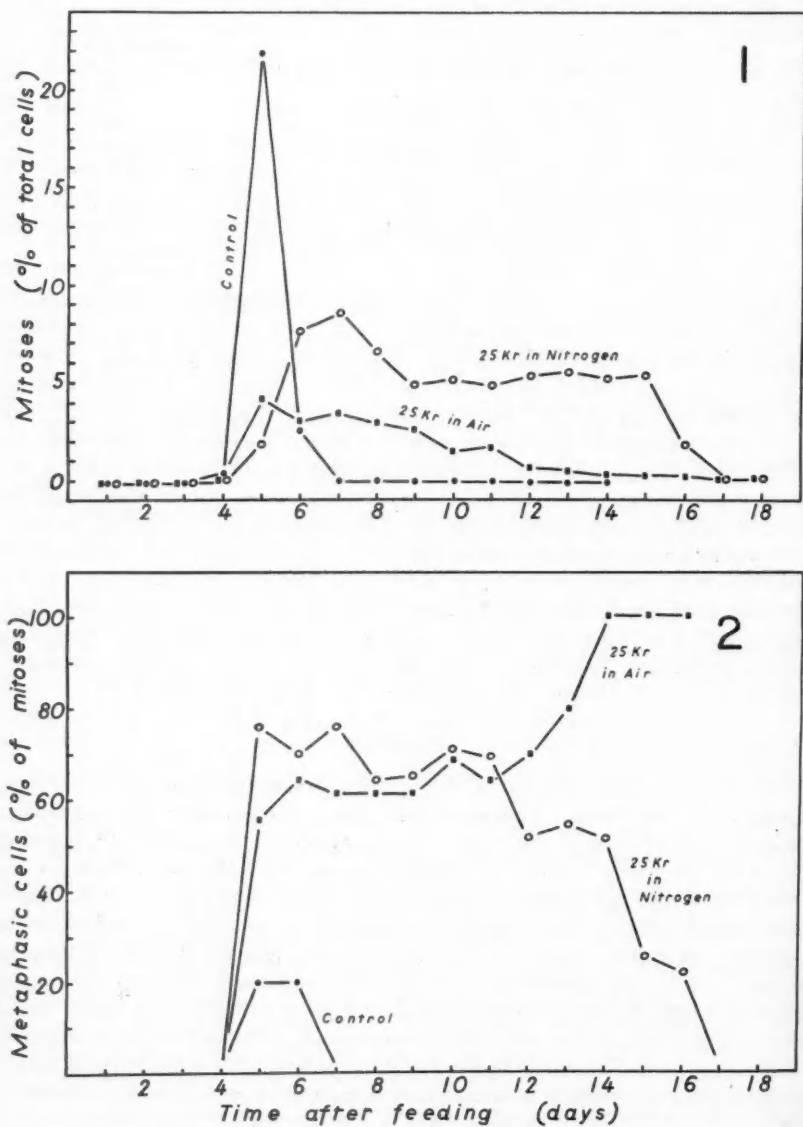
### Methods

In *Rhodnius*, cell division in the epidermal layer of fourth stage nymphs prior to the formation of a new cuticle begins 5 days after engorgement on blood. Cell division in the epidermis is initiated by the action of a hormone from the thoracic gland (4); to avoid damage to these glands, the thoracic and head regions of the nymphs were shielded in these experiments, and only the projecting abdomen of each insect was exposed through a 5-mm hole in a lead shield 5 cm in thickness. The dose administered with a 2-Mev X-ray machine could not be determined accurately, but measured without the shield, amounted to 25,000 r (at 16 cm from the target at a dose rate of 6000 r/min.). For 10 minutes before and during irradiation, nitrogen or air was passed through a small chamber containing the insects. All experimental insects were held continuously in a constant temperature and humidity cabinet at 25° C and 75% R.H. At daily intervals after feeding (the insects were fed

<sup>1</sup>Manuscript received September 8, 1959.

Contribution from Atomic Energy of Canada Limited, Chalk River, Ontario.

Issued as A.E.C.L. No. 907.



FIGS. 1 and 2. Percentage of total cells in mitosis (Fig. 1) and of dividing cells in metaphase (Fig. 2) at different days after irradiation and feeding.



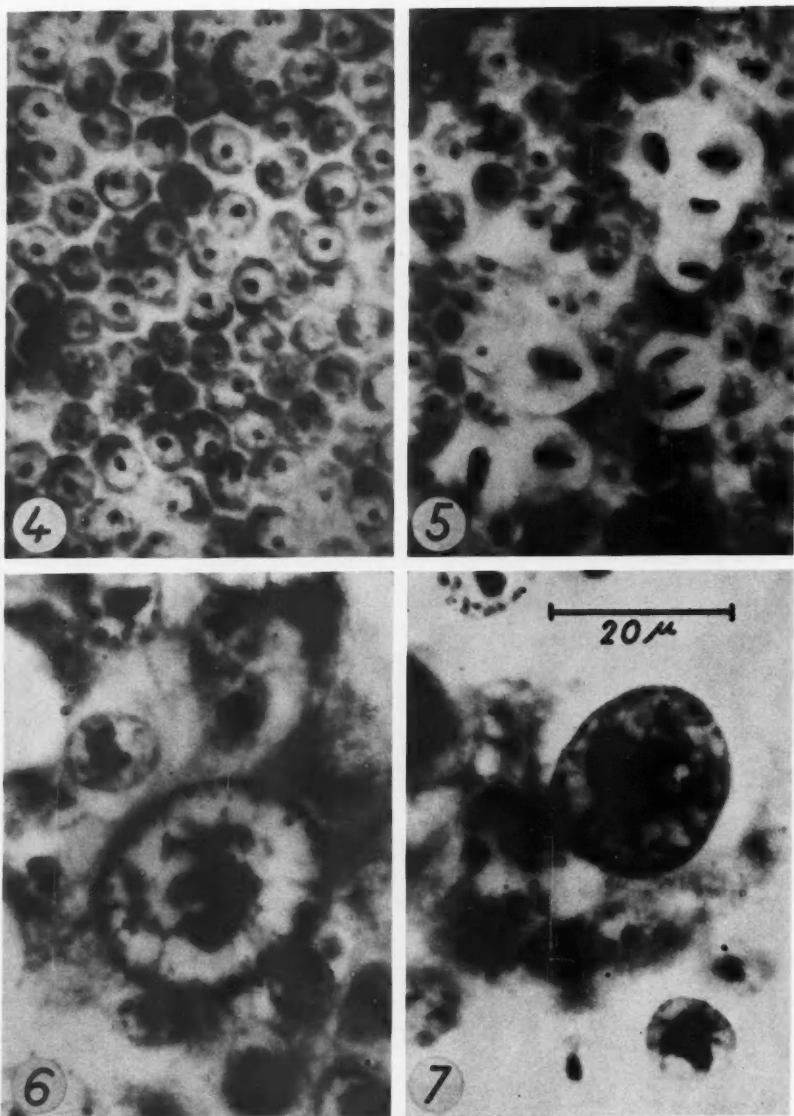


FIG. 4. Normal daughter cells produced in unirradiated tergites.

FIG. 5. Cells on tergite irradiated in nitrogen (25,000 r) at 12 days after feeding. Fewer cells with numerous metaphase stages (and others) present.

FIGS. 6 and 7. Sparse abnormal cells remaining on tergite after irradiation in air (25,000 r) and after majority of epidermal cells had been destroyed at metaphase. Magnification approximately 675 $\times$  in all cases. Stain, Lillie's haemalum; photographs taken at 12 days after feeding.

immediately after irradiation), whole tergites (the body wall of the dorsal abdomen) were prepared for cytological examination with Carnoy's fixative (6 parts ethanol, 3 parts chloroform, 1 part acetic acid) and Lillie's haemalum stain (5). Total cell counts were made with a square-field calibrated micrometer eyepiece at a magnification of 1000 times (counts based on a mean from 30 fields); all observations were made on the central position of the sixth abdominal segment. In each case, mitotic counts were calculated from a total of 2500 cells.

### Results

Except for the appearance, in the irradiated nymphs, of a few more degenerating cells (of a type also found in normal insects), development in the epidermal cells during the first 4 days after feeding was identical in the irradiated and the control insects. Mitosis began on the fifth day, and in the controls a sharp peak in the number of cells undergoing division was found at 5 days (Fig. 1); division was completed by the sixth or seventh day. In the irradiated groups, mitosis was initiated at the same time, but in comparison with controls, mitotic figures never became as plentiful, and they persisted for more extended periods. After an initial rise to only 4% incidence of mitosis on the fifth day, it gradually declined in the insects irradiated in air; in nymphs exposed in nitrogen, the proportion of cells in division increased to about 8 per cent on the 7th day and persisted in appreciable numbers until a sharp decline on the 15th and 16th days.

Cells irradiated before the onset of division generally passed through the early stages of division but were arrested for prolonged periods in metaphase. Although some degree of delay was observed in other stages (Table I), this unusual behavior represented a very large part of the over-all delay, as shown in Fig. 2. In the controls, the proportion of metaphase figures did not rise to more than 20% of the total cells in mitosis; the rapidity with which the unirradiated cells passed through this stage accounts for their scarcity. On the other hand, the higher proportion of cells observed at metaphase in the irradiated group is due to the extended delay at this stage of division. In the cells treated with nitrogen during irradiation, mitosis resumed after the delay, and the rapid decrease in numbers after 12 days showed that the cells had passed through division. Development beyond metaphase did not occur in insects irradiated in air, and the percentage of dividing cells in metaphase steadily increased to 100% (Fig. 2). Owing to cell destruction during this stage, the total number of cells present with this treatment had decreased by the 17th day from about 9500 to about 2000 per sq. mm, all in an abnormal condition.

The total number of cells in the preparations shows the final result of mitotic inhibition and cell destruction (Fig. 3). The numbers of cells in controls is more than doubled by the seventh day, by which time all epidermal cells had completed mitosis. The increase in cell number from 9000 to 24,000/sq. mm would seem to indicate that some cells might divide more than once; however, in view of the short period during which divisions can be observed it seems impossible that any cells could pass through two mitotic divisions, and

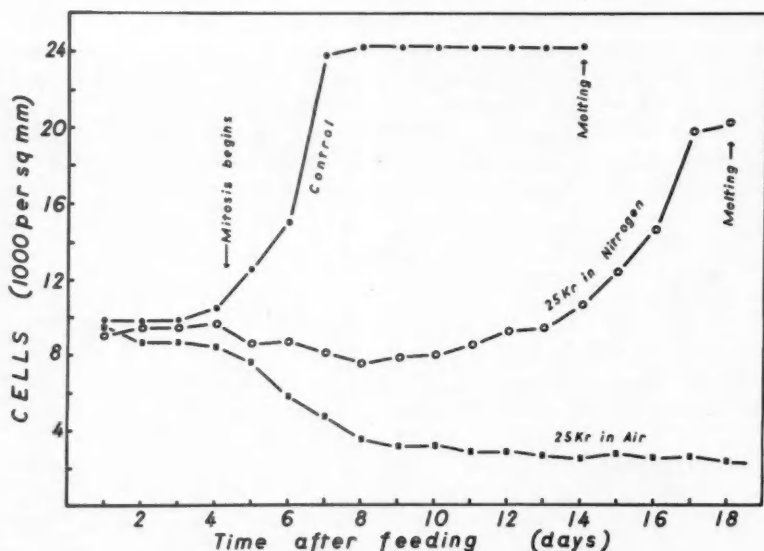


FIG. 3. Cell count at different days after irradiation and feeding.

the increase must be the result of the compression of the new cell layer under the old cuticle. The total number of epidermal cells in insects irradiated in air declined steadily as many disintegrated in metaphase, and molting never occurred, simply because of the lack of functional cells. Irradiation in nitrogen at the same dose allowed most of the cells to undergo a prolonged division, and the numbers eventually increased to a point where a new cuticle could develop. Molting took place on the 18th day, 4 days later than in controls.

Significant delay occurred in all stages of mitosis in insects irradiated in nitrogen (Table I). Many of the cells irradiated in air reached metaphase; a very few anaphase figures appeared, and these only on the fifth and sixth days.

The condition of the epidermal layer cells in the three groups at 12 days after feeding is shown in Figs. 4-7. After this period mitosis in normal insects has produced a multitude of daughter cells (Fig. 4), while in those irradiated in nitrogen the cells were less plentiful with metaphase figures (and others) still present (Fig. 5). The cell layer irradiated in air (Fig. 6 and 7), in which destruction occurred during metaphase, has still fewer cells, all in an abnormal condition. Giant cells like those seen in these preparations did not appear in earlier experiments (3), where high doses administered to small areas killed all exposed cells.

### Discussion

The delay in the completion of mitosis caused by the latent effects of radiation is readily observable in *Rhodnius* epidermis owing to the degree of

TABLE I

Development of dividing cells at successive days after irradiation (in nitrogen and air) and feeding

| Number<br>of days<br>after<br>feeding | % of all cells in: |           |          |           | % of<br>cells in<br>mitosis | % of<br>dividing<br>cells in<br>metaphase |
|---------------------------------------|--------------------|-----------|----------|-----------|-----------------------------|---|
|                                       | Prophase           | Metaphase | Anaphase | Telophase |                             |   |
| Controls                              |                    |           |          |           |                             |   |
| 4                                     | 0.1                | —         | —        | —         | 0.1                         | —   |
| 5                                     | 15.1               | 4.4       | 1.5      | 0.7       | 21.7                        | 20  |
| 6                                     | 1.2                | 0.5       | 0.4      | 0.4       | 2.5                         | 20  |
| 14                                    | Molt               |           |          |           |                             |   |
| 25 kr in nitrogen*                    |                    |           |          |           |                             |   |
| 5                                     | 0.4                | 1.2       | —        | —         | 1.6                         | 75  |
| 6                                     | 1.8                | 5.2       | 0.4      | 0.1       | 7.5                         | 70  |
| 7                                     | 1.1                | 6.5       | 0.5      | 0.5       | 8.6                         | 75  |
| 8                                     | 1.4                | 4.2       | 0.6      | 0.4       | 6.6                         | 64  |
| 9                                     | 1.4                | 3.2       | 0.1      | 0.2       | 4.9                         | 65  |
| 10                                    | 0.8                | 3.7       | 0.2      | 0.4       | 5.1                         | 72  |
| 11                                    | 1.1                | 3.4       | 0.3      | 0.3       | 5.0                         | 68  |
| 12                                    | 2.0                | 2.8       | 0.2      | 0.4       | 5.4                         | 52  |
| 13                                    | 2.1                | 3.1       | 0.2      | 0.3       | 5.7                         | 54  |
| 14                                    | 2.9                | 2.7       | 0.4      | 0.3       | 5.3                         | 51  |
| 15                                    | 2.7                | 1.8       | 0.5      | 0.5       | 5.4                         | 23  |
| 16                                    | 1.2                | 0.4       | 0.2      | 0.1       | 1.9                         | 21  |
| 17                                    | —                  | —         | 0.1      | —         | 0.1                         | —   |
| 18                                    | Molt               |           |          |           |                             |   |
| 25 kr in air*                         |                    |           |          |           |                             |   |
| 5                                     | 1.3                | 2.4       | 0.5      | —         | 4.2                         | 57  |
| 6                                     | 0.9                | 1.7       | 0.1      | —         | 2.7                         | 65  |
| 7                                     | 1.4                | 2.3       | —        | —         | 3.7                         | 62  |
| 8                                     | 1.1                | 1.8       | —        | —         | 2.9                         | 62  |
| 9                                     | 0.9                | 1.5       | —        | —         | 2.4                         | 62  |
| 10                                    | 0.4                | 0.9       | —        | —         | 1.3                         | 69  |
| 11                                    | 0.6                | 1.1       | —        | —         | 1.7                         | 65  |
| 12                                    | 0.2                | 0.5       | —        | —         | 0.7                         | 71  |
| 13                                    | 0.1                | 0.4       | —        | —         | 0.5                         | 80  |
| 14                                    | —                  | 0.2       | —        | —         | 0.2                         | 100                                       |
| 15                                    | —                  | 0.1       | —        | —         | 0.1                         | 100                                       |
| 16                                    | —                  | 0.1       | —        | —         | 0.1                         | 100                                       |
| 17                                    | —                  | —         | —        | —         | —                           | —   |
| 18                                    | No molt            |           |          |           |                             |   |

\*Insects fed immediately after irradiation.

synchrony that characterizes division following a blood meal. Earlier results with radiation burns (2) suggested a protective effect of oxygen deficiency during irradiation (in cells in the area surrounding the small burn). The present observations confirm this conclusion and serve also to indicate the unique nature of the mitotic inhibition, this being associated with an arrested metaphase stage.

It is clear from these studies that X irradiation did not interfere greatly with the initiation of mitosis. Although some irradiated cells lingered in prophase, we did not find any evidence of reversion of middle and late prophase

nuclei to earlier stages as reported by Gaulden *et al.* (6) in grasshopper neuroblasts. The greatest mitotic inhibition occurred during metaphase, and it was significant that even when irradiated at the high doses in air in these experiments, many cells could still proceed to metaphase. The delay in metaphase represented a large part of the total time required to complete division and resulted in a piling-up of observable metaphases until as many as 60% of the epidermal cells were in this stage at 8 or 10 days after feeding. In this connection, it may be significant that the Hemiptera, as a group, possess chromosomes with a diffuse spindle attachment (7), which might make these insects highly sensitive to metaphase delay. Nebel (8), on the other hand, has found a similar delay at metaphase in irradiated mouse spermatocytes.

In our studies, significant metaphase delay has occurred at doses as low as 2000 r after an exposure in air. At the higher dose used in this work, oxygen deficiency during irradiation allowed the completion of division; subsequent tests have shown that 9000 r in air produced the same cytological effect as 25,000 r in nitrogen. This "air-nitrogen ratio" of 2.8 agrees with that observed for mitotic inhibition in bean root (9), and for the size of burns and molting times in *Rhodnius* (2).

### Acknowledgments

The authors wish to acknowledge the help of L. G. Hunter in fabricating various items of equipment used in this study.

### References

1. HOLLAENDER, A. High energy radiation. Vol. I. *In* Radiation biology. McGraw-Hill Book Co. Inc. 1954.
2. BALDWIN, W. F. and SALTHOUSE, T. N. Oxygen deficiency and radiation damage in *Rhodnius*. *Nature*, **183**, 974 (1959).
3. BALDWIN, W. F. and SALTHOUSE, T. N. Latent radiation damage and synchronous cell division in the epidermis of an insect. I. Nonreversible effects leading to local radiation burns. *Radiation Research*, **10**, 387-396 (1959).
4. WIGGLESWORTH, V. B. The physiology of insect metamorphosis. Cambridge University Press, London. 1954.
5. LILLIE, R. D. Histopathologic technic and practical histochemistry. The Blakiston Co. 1954.
6. GAULDEN, M. E., NIX, M., and MOSHMAN, J. Effects of oxygen concentration on X-ray-induced mitotic inhibition in living *Chortophaga* neuroblasts. *J. Cellular and Comp. Physiol.* **41**, 451-469 (1953).
7. HUGHES-SCHRADER, S. and RIS, H. The diffuse spindle attachment of coccids, verified by the mitotic behaviour of induced chromosome fragments. *J. Exptl. Zool.* **87**, 429-456 (1941).
8. NEBEL, B. R. and MURPHY, C. J. Damage and recovery of mouse testis after 1000 r acute localized irradiation, with reference to restitution cells, Sertoli cell increase, and type A spermatogonia recovery. *Radiation Research*. In press (1959).
9. EVANS, H. J., NEARY, G. J. and TONKINSON, S. M. Mitotic delay in irradiated roots of *Vicia faba*: the effect of oxygen. *Nature*, **181**, 1083 (1958).

## A REVISION OF THE NEARCTIC EXENTERINI\* (HYMENOPTERA: ICHNEUMONIDAE)

### III. EXYSTON SCHIØDTE<sup>1</sup>

W. R. M. MASON

#### Abstract

The genus *Exyston* and its 20 Nearctic species are discussed. Ten new species are described and nine previously described species are reduced to synonymy. Two species have proved unrecognizable.

#### *Exyston* Schiødte

*Exyston* Schiødte, 1839, in Guérin, Mag. Zool. Ins. 9, 12.

Type: *Ichneumon cinctulus* Gravenhorst. Monobasic.

*Anecphysis* Foerster, 1868, Verhandl. Naturhist. Ver. Rheinlande, 25, 195.

Type: (*Anecphysis curvineura* Davis) = *Exyston marginatus* Provancher.

Included by Davis, 1897, Trans. Am. Entomol. Soc. 24, 236.

*Tricamptus* Foerster, 1868, Verhandl. Naturhist. Ver. Rheinlande, 25, 195.

Type: *Cteniscus* (*Tricamptus*) *pratorem* Woldstedt.

Included by Woldstedt, 1877, Bull. Acad. Sci. St. Petersburg, 23, 454.

The synonymy of *Tricamptus* has been discussed by Kerrich, 1952 (1). I am using *Anecphysis* subgenerically for those species of *Exyston* characterized by vomeriform third valvulae.

Almost all species of *Exyston* may be distinguished from other Exenterini by the petiole, which is auriculate and over twice as long as its greatest width (the few short-petioled specimens may be distinguished from *Smicroplectrus* by the doubled subtegular ridge, spurless hind tibiae, and simple tarsal claws, as well as by general habitus).† In addition the apical abdominal tergites are strongly arched, the subgenital plate is reduced and weakly sclerotized, the ovipositor is thin and sharply pointed, and the egg has only a single stalk.

The closest relative is *Smicroplectrus*. The relationships are discussed in Part II (3).

The genus divides naturally into two subgenera, *Exyston* and *Anecphysis*. The more primitive, *E.* (*Anecphysis*), includes the groups of *marginatus*, *variatus*, and *speciosus*. They are characterized by vomeriform third valvulae, hind tibia without any apical projection, mesonotum without any tectiform ridge along the inner side of the marginal flange. All the old world species except *cinctulus* belong here.

The other subgenus, *E.* (*Exyston*), includes the groups of *cinctulus*, *venustus*, and *flavens*. They are characterized by having simple third valvulae. All

\*Manuscript received July 18, 1959.

Contribution from Taxonomy Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Canada.

\*The International Congress of Zoology in 1958 voted to base family group names on priority at the supergeneric level and not on the oldest included genus. Accordingly the name formerly used for this tribe (Parts I and II, Cteniscini) is replaced by Exenterini, dating from Foerster, 1868 (Verhandl. Naturhist. Ver. Rheinlande, 25, 194).

†See key to genera, Part I (2).

but the *cinctulus* group have an apical projection on the hind tibia and a tectiform ridge along the inner side of the marginal flange of the mesonotum opposite the base of the forewing. The distribution is dominantly Nearctic.

Head transverse; clypeus one-third to one-half as long as wide; inner margins of eyes subparallel to divergent below; malar space more than basal width of mandible; temples about equal or slightly wider than eyes, convergent behind eyes; occipital carina usually joining hypostomal carina above its lower end, sometimes obsolescent or absent at its lower end, strong, and not meeting hypostomal carina in one Nearctic species but bizarrely modified in some Palaearctic species.

Notaulices present at cephalic end only; mesonotal flange and axillary tongue well developed; a tent-like ridge, usually open posteriorly, present just mesad of the mesonotal flange in some species; subtegular ridge doubled posteriorly (Part I, Fig. 4). Propodeum short, rounded, or somewhat declivate, fully but never very strongly carinate. Hind tibiae without an apical projection in most species; tarsal claws not pectinate except in two species (*flavens* and *pratorum*), but often with stout ventral hairs that resemble pectination.

Petiole auriculate, 1.3 to over 3 times as long as its greatest width, but seldom less than twice as long as wide; sides subparallel to weakly divergent. Abdomen usually strongly clavate, the apical tergite as deep as or deeper than wide; subgenital plate reduced and inconspicuous. Third valvulae fundamentally vomeriform, but in the subgenus *E. (Exyston)* apparently secondarily appressed ventrally and thus appearing simple. Ovipositor shorter than petiole, never projecting beyond apex of abdomen, straight, sinuate, or curved upward, usually tapering to a sharp point and with small teeth or none.

Egg usually ovate and with a long, single, apical stalk but sometimes reniform and with a short, midventral stalk.

*Exyston* occurs throughout the Holarctic region except in arctic and desert areas. It is the only genus of the tribe which occurs in any numbers in the grasslands and semiarid regions of North America. Most species are to be found in grassy places and are probably parasitic on sod-feeding larvae of Dolerinae (Tenthredinidae), but the only species reared in North America is a parasite of a *Vaccinium*-feeding nematine (Tenthredinidae).

#### KEY TO NEARCTIC SPECIES OF EXYSTON

1. Occipital carina hairy and crenulate, strong and complete, but separated from, and subparallel to, the hypostomal carina; outer apical margin of antennal scape deeply excavated (subgenus *Exyston*, *cinctulus* group)..... *clavatus* (Cress.) 2  
 Occipital carina neither hairy nor crenulate, either incomplete at its lower end or meeting the hypostomal carina at a sharp angle; outer apical margin of antennal scape broadly and shallowly excavated.....
2. Ovipositor valves simple, not vomeriform; apex of hind tibia with a small, externo-ventral projection; mesonotum with the lateral marginal groove near the wing base filled and not foveolate, often with a raised, open-ended ridge (Fig. 21) (subgenus *Exyston*, *flavens*, and *venustus* groups)..... 3  
 Ovipositor valves vomeriform, i.e. strongly divergent below, and hairy internally; apex of hind tibia unarmed; lateral marginal groove of mesonotum normal, deep and foveolate (subgenus *Anecphysis*)..... 9

3. Face very short, about 2.4 times as wide as long (Fig. 19); tarsal claws pectinate; cheeks in frontal aspect strongly convergent at about 80° (Fig. 20); cheeks in profile strongly convex; small, stocky, polished, black and yellow, eastern species (*flavens* group)..... *flavens* Ds.  
Face long, less than twice as wide as long (Fig. 18); tarsal claws simple; cheeks in frontal aspect weakly convergent at 45° to 60° (Fig. 17); cheeks in profile concave, flat, or weakly convex; larger, slim, mostly western species (*venustus* group)..... 4
  4. Costulae completely absent; body highly polished and very sparsely punctate, especially on mesopleuron..... 5  
Costulae present, sometimes weak but rarely absent; body punctuation normal, the mesopleuron conspicuously punctate..... 6
  5. Occipital carina complete; cheeks strongly concave; antennal joints as wide as apex as at middle; auriculae of petiole large and pointed..... *politus* Davis  
Occipital carina usually incomplete at lower end; cheeks usually weakly concave; apical joints of antenna only 0.7 times as wide as middle joints; auriculae of petiole not pointed nor unusually large..... *venustus* (Cress.)
  6. Cheeks in profile distinctly convex near lower end of eye; occipital carina of uniform height to its junction with hypostomal carina; mesonotal ridge open-ended, large, and sharp-topped (Fig. 21)..... *humeralis* Ds.  
Cheeks in profile flat or concave near lower end of eye; occipital carina weaker or absent at lower end; mesonotal ridge often low and not open-ended..... 7
  7. Mesonotal ridge tall and deeply open at the posterior end; range entirely east of Rocky Mountains..... *illinois* n. sp.  
Mesonotal ridge usually low or flat, and usually not open-ended (if opening is present it is no deeper than its own width); range mostly western..... 8
  8. Mesonotum and propodeum entirely black..... *chamaeleon* n. sp.  
Mesonotum of female with a pair of longitudinal yellow stripes; male with yellow lateral markings on propodeum..... *excelsus* (Cress.)
  9. Build generally short and stocky (Fig. 22) (Tables I and II) (*marginatus* group)..... 10  
Build longer and slimmer (Tables I and II)..... 11
  10. Color of body black and yellow, propodeum never red..... *marginatus* (Prov.)  
Color of body mostly red, propodeum always red..... *tectus* n. sp.
  11. Cheek near lower margin of eye decidedly convex in profile, very rare west of the Rocky Mountains (*variatus* group)..... 12  
Cheek near lower margin of eye flat or nearly flat in profile, unknown east of Minnesota (*speciosus* group)..... 15
  12. Hind coxae, cheeks, postgenae, and thorax black, antennae yellow-tipped..... *boreotis* Davis  
Cheeks and postgenae yellow below; thorax often partly or mostly red, hind coxae red..... 13
  13. Cheeks broad and weakly convergent behind eyes; large, coarsely sculptured species; antennae yellow-tipped..... *hadros* n. sp.  
Cheeks narrower and more strongly convergent behind eyes; smaller, finely sculptured species; antenna almost always black-tipped..... 14
  14. Orbits usually complete and yellow, but if incomplete the gap is red; ovipositor uniformly curved upward in its apical half (Fig. 7); range from the Great Smoky Mountains to Massachusetts..... *austelli* n. sp.  
Orbits rarely complete and yellow, usually black behind; ovipositor straight and slightly decurved or sinuate (Fig. 1); widely distributed..... *variatus* Prov.
  15. Occipital carina absent below level of middle of eye..... *speciosus* Davis  
Occipital carina complete..... 16
- (The following four couplets are based on female specimens only)
16. Ovipositor apically thick and decurved, with a few dorsal spines (Fig. 5); egg reticulate, long-ovate, stalk terminal; prepectus and mesosternum of female almost entirely black..... *spinulosus* n. sp.  
Ovipositor apically sharply pointed and without spines; egg stalk terminal or mid-ventral; prepectus and mesosternum of female usually extensively yellow..... 17

17. Ovipositor strongly curved upward in apical third (Fig. 10); egg reticulate, short-ovate, stalk terminal.....*californicus* n. sp.  
Ovipositor more or less straight, egg otherwise shaped..... 18
18. Egg ovate, stalk terminal; ovipositor weakly sinuate (Fig. 3); red color of abdomen often reduced or absent; cheek and postgena black.....*ater* n. sp.  
Egg reniform, stalk midventral; abdomen usually mostly red; cheek and postgena yellow below, sometimes narrowly black next to hypostomal carina..... 19
19. Egg with an apical sagittal flange; ovipositor weakly curved upward apically (Fig. 9); mesosternum and prepectus almost entirely black; hind coxae black or red, never yellow below.....*lophotos* n. sp.  
Egg without any flange; ovipositor weakly sinuate and decurved apically (Fig. 8); mesosternum and prepectus extensively yellow; hind coxa yellow below apically.....*reniformis* n. sp.

TABLE I

Distinctions between *E. variatus* and *E. tectus* in eastern Canada and northeastern U.S.A.

| Character          | <i>Exyston variatus</i>   | <i>Exyston tectus</i>  |
|--------------------|---|--|
| Clypeus            | Weakly convex in profile; slightly longer (Fig. 12)   | Strongly convex in profile; slightly shorter (Fig. 11)   |
| Face               | Usually centrally raised in a squarish, flattened, densely, and coarsely punctate area that is fairly sharply differentiated from the sides | Raised centrally in a rounded, densely punctate area that is not sharply differentiated from the sides |
| Antennae           | As long as, or longer than, the body; almost always black-tipped  | Shorter than the body; tips and apical half or more unicolorous  |
| Cheeks and temples | Weakly convergent behind eyes in dorsal aspect (Fig. 14)  | More strongly bulging; not convergent behind eyes (Fig. 12)  |
| Eyes               | Larger; in dorsal aspect as wide as temples and projecting laterally  | Smaller; in dorsal aspect not as wide as temples and scarcely projecting beyond them                   |
| Hind coxae         | Longer and slimmer  | Shorter and more globular  |
| Propodeum          | Profile in lateral aspect rounded; as high as long  | Profile in lateral aspect more strongly declivous; higher than long (Fig. 22)                          |
| Second tergite     | Length is 1.25 to 2.0 times width of apex; sides less divergent   | Length subequal to width of apex; sides more divergent   |

TABLE II

Distinctions between *E. marginatus* and *E. ater* in the Puget Sound area

| Character  | <i>Exyston marginatus</i>                       | <i>Exyston ater</i>                             |
|------------|---|---|
| Cheeks     | Distinctly convex near lower end of eyes        | Flat near lower end of eyes                     |
| Antennae   | About as long as body                           | Shorter than the body                           |
| Scutellum  | Basally punctate                                | Basally rugulose or rugulopunctate              |
| Hind coxae | Shorter and more globular                       | Longer and slimmer                              |
| Abdomen    | Less than 3 times as long as its greatest width | More than 3 times as long as its greatest width |

NOTE: The characters in these two tables may be used, with a lesser degree of reliability, to separate species of the *marginatus* group from those of the *speciosus* and *variatus* groups.

***Exyston (Anecphysis) Davis***

## MARGINATUS GROUP

This group includes the Nearctic *marginatus* and *tectus*. The Palaearctic *pratorum* also falls here. The European species *phaeorrhæus* (Hal.) and *subnitidus* (Grav.) probably belong here or near here and *calcaratus* Thom. seems to be closely related.

The species included are characterized by a short, small, compact, and stout habitus compared to other groups of the genus (Tables I and II).

***Exyston (Anecphysis) marginatus* Provancher**

*Exyston marginatus* Provancher, 1886, Addit. Faune Canada, Hymen., p. 99 (o.d., ♀, Toronto, Ont., type lost).

*Anecphysis curvineura* Davis, 1897, Trans. Am. Entomol. Soc. 24, 234 (o.d., ♀, N.Y., type in ANSP).

*Exyston nigreo* Davis, 1897, Trans. Am. Entomol. Soc. 24, 236 (o.d., ♂, Colo., type in ANSP).

In 1951 (4) I incorrectly synonymized this species with *Exyston excelsus* (Cresson). There are several discrepancies in coloration, especially of the legs, between *excelsus*, or any of the segregates of that species, and Provancher's description. I have now several specimens from the Toronto district: the males agree perfectly with the Provancher description and belong to the species heretofore known as *E. nigreo* Davis.

This species is morphologically indistinguishable from *E. tectus* but may be very easily separated by the invariably black and yellow color pattern: that of *tectus* being always at least partly red. The range of *marginatus* in its southwestern parts (British Columbia, Washington) overlaps that of *E. ater*. Both species were taken at Mt. Rainier, Wash., by the Townes family, but *marginatus* flew mainly at higher altitudes and later in the season than *ater*. Many specimens of these species have identical color patterns but may be distinguished by the characters summarized in Table II.

*Female*

Length 4.5 to 8 mm; head not elongated (Figs. 11, 12); external apical margin of antennal scape shallowly excavated; face about 2.2 times as wide as long, cheeks in frontal aspect converging at about 80°; cheek strongly to very weakly convex near bottom of eye, but making an ill-defined angle of about 120° with postgena; occipital carina situated at the angle, curving usually evenly to hypostomal carina and meeting it at an angle of about 80°, of uniform width throughout.

Mesonotum without any lateral ridge; propodeum completely carinate, rounded, and elevated above, but short and declivate behind; scutellum moderately coarsely punctate, about as long as wide; apex of hind tibia without any projection.

Petiole in dorsal aspect with sides subparallel; auriculae weakly developed, rounded; ovipositor valves vomeriform; ovipositor sharply pointed and weakly decurved at extreme apex.

Color of head, thorax, and abdomen black with the following parts yellow: mouth parts, clypeus, face, inner orbit usually to near top of eye, lower third of cheek, propleuron, lower margin of pronotum laterally, prepectus behind anterior coxae, sometimes adjoining parts of mesosternum, and mesopleuron, tegula, wing base, subtegular ridge, scutellum, postscutellum, genitalia, venter of abdomen except for petiolar sternite; medioapical margins of tergites, the first three or four being narrow, those caudad becoming increasingly broad and extending further laterad with tergite seven, or occasionally tergites six and seven, entirely yellow. A narrow, diffuse, reddish band rarely interposed between black and yellow parts of second to fourth tergites. Antenna reddish orange, black above and basally; scape usually yellow below. Four anterior coxae and trochanters yellow; remainder of these legs red, the femora, and sometimes tibiae, yellow in front; femora, especially in northern specimens, often black behind. Hind coxa black; hind trochanter yellow. Hind femur black to red: usually black in northern specimens; usually red with black annuli at base and apex in eastern specimens. Hind tibia black or dark brown, with basal and broad central yellow annuli; hind tibia brown. Wing often weakly infumated, stigma black with base yellow, other veins dark brown.

#### *Male*

Morphologically similar to female. Yellow areas less extensive than those of female as follows: most northern specimens with two broad, vertical, black bands on face; sometimes entire central area of face black; western specimens often with upper part of face black; cheek black, or yellow only to bottom of eye; thorax black except for tegula, scutellum, and postscutellum; subtegular ridge and lower posterior corner of pronotum occasionally yellow. Apical bands of abdominal tergites of uniform width on all tergites, being 0.5 to 0.2 times as wide as length of tergite; apical markings on tergites one and six sometimes absent; tergite seven never with an apical yellow margin; apical tergites, and sometimes genitalia, dark brown to black. Antenna often dark brown; four anterior coxae sometimes basally, or, rarely, entirely, black.

#### *Egg*

Ovate; stalk apical; surface smooth but sometimes with faint reticulation.

#### *Variation*

Two specimens, a male and a female, from the San Francisco area of California show an unusual development of xanthism although morphologically they are within the normal range of variation of the species. In particular they have lateral yellow spots on propodeum; apical yellow bands of tergites occupying about half the segment; four anterior legs entirely yellow except for black basal inner surfaces of the femora; hind femur black, and hind tibia yellow, except the apical 0.2. The male is even more strongly xanthic; half or more of each abdominal tergite yellow, forelegs entirely yellow, and hind coxa yellow apically.

*Specimens Seen* (about 100 ♂♂ and ♀♀)

*Holotype*.—? Male, Toronto, Ontario, Brodie (type lost).

*Other specimens*.—ALASKA: Mt. McKinley National Park, Summit Lake in Isabella Pass at 3300 ft, Big Delta; N.W.T.: Norman Wells, Yellowknife, Cameron Bay on Great Bear Lake; B.C.: Agassiz, McGillivray Creek Game Preserve near Chilliwack; WASH.: Elbe, Mt. Rainier at 4700 to 5300 ft, Snoqualmie Pass, Seattle, Westport; OREG.: Cannon Beach; CALIF.: Crescent City, Elkhorn Ferry in Yolo Co., Sand Dunes at San Francisco; ALTA.: Banff, Gull Lake; SASK.: Saskatoon; COLO.: Rabbit Ears Pass at 9500 ft, Morley, Grizzly Creek, Copeland Park in Boulder Co., Big Muddy Creek at 7500 ft, near Kremmling; MINN.: Itasca Park, Jenkins; MAN.: Gillam, Minnedosa; MICH.: Midland Co., ONT.: Mildmay, Toronto, Ottawa; QUE.: Fort Chimo, Great Whale River, Forrestville, Quebec, Cascapedia; MAINE: West Hamden; N.B.: St. John; N.S.: Truro; P.E.I.: Alberton, Parrsboro; MASS.: Cummington; N.Y.: Derby, Ithaca, Buffalo; PA.: Northeast; N.C.: Highlands at 3300 ft, between Blowing Rock and Linville.

This species occurs transcontinentally from the Hudsonian to the Transition zones but is not known from the Aleutian or Bering Sea regions. All the specimens I have collected were swept from willows. In the north the species flies (as do most Ichneumonidae) in midsummer, but in the Transition zone there may be two generations, specimens being taken in May-June and again in August-September.

***Exyston (Anecphysis) tectus* n. sp.**

This is closely related to *E. marginatus* Provancher and is not distinguishable by morphological characters but only by color pattern. However, it has a more southern range than *marginatus*, and in the broad area where the two are sympatric, there is not the slightest evidence of intergradation. In fact, I collected typical specimens of *marginatus* at the same time and place as the type series of *tectus*. Authors in the past have failed to distinguish this from the commonest eastern species, *E. variatus* Provancher. They may be distinguished by the characters summarized in Table I (Fig. 22).

*Holotype*

Female; length 6 mm; head not elongated (Figs. 11, 12); external apical margin of antennal scape shallowly excavated; face about 2.2 times as wide as long; cheeks in frontal aspect converging at about 80°; cheek strongly convex near bottom of eye, but making an ill-defined angle of about 120° with postgena; occipital carina situated at the angle, curving rather evenly to hypostomal carina, and meeting it at an angle of about 80°, of uniform width throughout.

Mesonotum without any lateral ridge; propodeum completely carinate, rounded above but shortened and declivate behind; scutellum moderately coarsely punctate; apex of hind tibia without any projection.

Petiole in dorsal aspect with the sides subparallel, auriculae weakly developed, rounded; ovipositor valves vomeriform; ovipositor sharply pointed and weakly decurved apically.

Color red with the following parts yellow: mouth parts, clypeus, face inner orbit nearly to top of eye, cheek to slightly above margin of eye, propleuron, tegula, wing base, scutellum, postscutellum, narrow diffuse apical margin of fourth tergite, diffuse apical quarter of fifth tergite, sixth and following tergites, venter of abdomen and genitalia. Black coloration confined to region around ocelli, frons medially, postocciput, lower medial part of pronotum, prepectus, mesosternum, sutures on dorsal part of thorax, central lobe of mesonotum anteriorly, metasternum, and basal segments of antenna. Antennal flagellum red except first joint; scape reddish below. All legs red except for trochanters, middle coxa apically, and anterior coxa; indefinite black annuli at base and apex of hind femur and base of hind tibia. Wing infumated, stigma dark brown with yellow base and yellowish marginal vein.

#### *Allotype*

Male; resembling female except for the following details: scape entirely red, face with two red vertical lines running from antennal sockets to clypeus, cheek almost entirely red, propleuron black, postscutellum and base of scutellum red, tergites of abdomen entirely red, hind coxa entirely red, anterior coxa red with yellowish apex.

#### *Egg*

Ovate, the surface with a very faint reticulation, almost smooth apically; stalk terminal.

#### *Variation*

*Female paratypes*.—Length 4.5–7.5 mm; unusually constant morphologically. Color varying little throughout most of range but specimens from extreme north showing melanism, red of the body being replaced by black except in the following areas: small reddish suffusions near wing bases, propodeum, apical spot on petiole, medioapical half of second tergite, generally strong red suffusion on third tergite, median red suffusion on fourth and fifth tergites. Legs except hind and middle coxae and most of antennal flagellum remaining red. Face sometimes suffused with red medially. Yellow areas sometimes more extensive than those of type as follows: inner orbit broadly to top of eye, cheek to half eye-height, lower margin of pronotum, subtegular ridge, prepectus and median part of mesosternum, metasternum red or yellow, front and middle femora and tibiae, scape below. Pronotum sometimes without any black marking; postgena sometimes red at lower extremity.

*Male paratypes*.—Length 4.5–7.5 mm; with very little morphological variation. Color varying similarly to that of female, melanic forms being commoner and the extreme melanic having cheek, central stripes of face, and propleuron black in addition to parts which are black in females. Yellow often more extensive than in allotype as follows: face entirely yellow, scape

yellow below, apical margins of third and following tergites yellow, front leg yellow except for femur behind, middle leg yellow in front.

*Specimens Seen* (about 240 ♂♂ and ♀♀)

*Holotype*.—Female, MacGillivray Creek Game Reserve near Chilliwack, British Columbia, July 23, 1953, W.R.M. Mason (CNC No. 6937).

*Allotype*.—Male, same data as above (CNC).

*Paratypes*.—B.C.: Chilliwack, Peat Bog at Pitt Meadows, Mission City, Agassiz, Canim Lake, Vernon, Oliver, Osoyoos, Robson, Fernie, Taylor; WASH.: Mt. Rainier at 5000 to 5500 ft, Snoqualmie Pass; OREG.: Corvallis; CALIF.: Big Flat on Coffee Creek in Trinity Co.; ARIZ.: near Alpine; N.M.: Beulah at 8000 ft; COLO.: Boulder, Lyons, Estes Park; WYO.: Big Horn Mountains near Buffalo, Sheridan Lake Camp in Yellowstone Park; ALTA.: Waterton Lakes, Gull Lake, Edmonton; SASK.: Snowden; MAN.: Riding Mountain; S.D.: 12 miles Southwest of Rapid City; MINN.: Itasca Park; IOWA: Butler Co.; MICH.: Midland Co., Wexford Co., Kalkaska Co., Montmorency Co.; ILL.: West Union, Charleston, White Heath, Makanda; OHIO: Bedford, Cleveland; ONT.: Ottawa, Simcoe, Constance Bay, Tweed, Bells Corners, Sudbury, Toronto; QUE.: Aylmer, Wright, Chelsea, Hemmingford, Brome, Georgeville, Montigny, Joliette, Kazabazua, Ste. Agathe des Monts, Montreal; N.B.: Charlotte Co., Waweg; P.E.I.: Brackley Beach; N.S.: Berwick, Kentville, White Point Beach, Petite Riviere; MAINE: Casco, Stratton; N.H.: Randolph, Mt. Washington at 5100 ft; VT.: Rutland, Jacksonville; MASS.: Boston, Brookline, Cummington, Winchendon, Rutland, Stow; CONN.: Voluntown, Hartford, Salisbury; R.I.: Kingston, Hopkington, Westerly; N.Y.: Cranberry Lake in Adirondak Mountains, Onondagua Co., Lockport, Ithaca, Bemus Pt., Stockport, Whitesville, Hancock, Oneonta, Poughkeepsie, Loch Muller in Essex Co., Slide Mountain in Ulster Co., Ausable Chasm; N.J.: Englewood, Moorestown, Ramsey; PA.: Spring Brook, Moosic; MD.: Takoma Park, Bowie; VA.: Falls Church, Skyline Drive, Great Falls; N.C.: Wake Co.; TENN.: Oak Ridge.

This is a common species through the Alleghenian Zone and extends into the cooler parts of the Carolinian. It is scarce across the central plains, where it has a narrow distribution along the southern edge of the boreal forest but occurs commonly in the Transition Zone of the western mountains.

In Maine, it has been reared from the willow-feeder *Pristiphora sycophanta* Walsh, and in Charlotte Co., N.B., from the blueberry-feeders *Pristiphora bivittata* (Nort.) and *P. idiota* (Nort.).

Both sexes are flying from May to October in the East, but the captures in the West are almost all made in early to middle summer. However, some August and September records from the Okanagan Valley of British Columbia (as well as May records) indicate a second generation there, too.

#### VARIATUS GROUP

This group includes the Nearctic *variatus*, *hadros*, *boreotis*, and *austelli*, and the Palearctic *albicinctus* (Grav.) and *genalis* Thom.

Species of this group are distinguished from the *speciosus* group by the rounded cheeks, especially at the level of the lower end of the eye.

They are distinguished from the *marginatus* group by the longer and slimmer habitus (Table I).

***Exyston (Anecphysis) variatus* Provancher**

*Exyston variatus* Provancher, 1877, Naturaliste can. 9, 15 (o.d., ♀, Cap Rouge, Que., type in MPQ).

*Exyston variatus* Provancher, 1879, Naturaliste can. 11, 249 (o.d., ♀, Cap Rouge, Que., type in MPQ).

*Exyston abdominalis* var. *rufinus* Davis, 1897, Trans. Am. Entomol. Soc. 24, 239 (o.d., ♀, N.H.).

This is the commonest member of the genus in Eastern North America and the most variable. It may be distinguished from *austelli*, *boreotis*, and *hadros* by the characters discussed under those species and from *tectus* by Table I.

This species seems to exhibit character displacement with *austelli*. Within the range of the latter species, *variatus* has a weakly sinuate ovipositor (Fig. 1) and very broadly incomplete outer and upper orbits. To the north and west, however, these characters become quite variable. The orbits are even complete in a few specimens from the Midwest and St. Lawrence Valley. The ovipositor varies from almost straight (Fig. 1, upper) to quite strongly sinuate (Fig. 1, lower) and I have even seen a pair of specimens from Alberta with ovipositor exactly like that of *austelli*. In other respects these specimens are typical of the dwarfed melanic populations characteristic of the northernmost parts of the range of *variatus*.

**Female**

Length 5 mm–9 mm; head not elongated (Figs. 13, 14); external apical margin of antennal scape shallowly excavated; face about 2.4 times as wide as long; cheeks in frontal aspect converging at about 70°; cheek convex and receding near lower margin of eye, curving abruptly onto the postgena; occipital carina present and complete, of uniform height throughout its length, meeting the hypostomal carina at an angle of 70°–80°; antenna weakly tapered toward base and apex.

Mesonotum with no lateral ridge; scutellum about as long as broad, smooth, and sparsely punctate anteriorly, more coarsely and densely punctate posteriorly, sometimes more coarsely sculptured throughout; propodeum completely carinate, rounded in profile; apex of hind tibia with no projection.

Petiole in dorsal aspect of uniform width throughout its length, but sometimes slightly broader apically; auriculae large and rounded; ovipositor valves vomeriform, ovipositor straight and sharply pointed, but sometimes sinuate, and usually slightly decurved at extreme apex (Fig. 1).

Color of head and thorax red or black, or variegated red and black, the following parts yellow: mouth parts, clypeus, face, inner orbit usually to near top of eye, but sometimes continued on vertex around top of eye, cheek

to one-third eye-height but sometimes higher and sometimes with a detached spot on outer orbit near top of eye, postgena to same height as cheek, propleuron, more or less of lower margin of pronotum, tegula, wing base, subtegular ridge, more or less of prepectus and adjacent parts of mesosternum and mesopleuron, mesosternum usually centrally, scutellum, postscutellum. Lower parts of mesothorax sometimes without yellow. Abdomen usually red but in northern specimens variegated with black. At maximum extent the following parts black: petiole, base of second tergite, sides of second and following tergites, fifth and sixth tergites basally. The following parts of the abdomen yellow: apex of fourth tergite narrowly or apices of third and fourth narrowly, apical half or more of fifth and following tergites and their lateral margins, venter and genitalia. Antennal scape black or red above, yellow below, pedicel black, basal half or less of flagellum black above, first flagellar joint usually black below, remainder of flagellum reddish to yellow with the apical one to five joints darkened or black, but in occasional individuals yellowish to the extreme apex. Four anterior coxae, trochanters, and femora in front, yellow; remainder of anterior legs light reddish. Hind coxa usually red with yellow below, but without yellow in specimens from the Apalachian region; extensively variegated with black in specimens from the Apalachian region and the northern fringes of the species range. Hind trochanter yellow, remainder of hind leg usually red but apices and bases of hind femur and tibia occasionally darkened. Wings usually hyaline but occasionally slightly infumated.

#### *Male*

Resembling the female morphologically except for averaging smaller in size and somewhat coarser in sculpture. The color is usually much darker than in the female, the yellow pattern differing as follows: yellow inner orbits not extending above top of eye, cheek yellow at the most only to bottom of eye. Yellow of thorax usually confined to tegula, subtegular ridge, scutellum, and postscutellum, but occasionally lower margin of pronotum and prepectal carina also yellow. Only narrow apical margins of abdominal tergites two to six yellow; abdomen sometimes without yellow. Head, thorax, and abdomen usually completely or extensively black except for yellow markings mentioned above. Ocellar triangle and mesosternum always black; bases of second and apical tergites usually black but always at least darkened. Color of antenna and leg about the same as in female, the hind coxa varying from entirely red to entirely black and often yellow below.

#### *Egg*

Ovate; stalk apical; surface sometimes reticulate.

*Specimens Seen* (about 250 ♂♂ and ♀♀)

*Holotype*.—♀, Cap Rouge, Quebec (MPQ).

*Other specimens*.—B.C.: Robson, ALTA.: Beaverlodge, Edmonton Wabamun, Bilby, Black Foot Coulee near Wainwright, Nordegg; SASK.: Prince Albert National Park, Harlan, White Fox; N.DAK.: Tower City;

MINN.: Ramsey Co., Cass Co., Cushing; WISC.: Sawyer Co., Madison, Dane Co.; ILL.: Urbana; IND.: Turkey Run; OHIO: Logan Co.; ONT.: Kearney, Toronto, Sudbury, Leamington, Ingersoll, Simcoe, Galetta, Eldorado, Fitzroy Harbor, Constance Bay, Ottawa; QUE.: Wakefield, Gracefield, Gatineau Park, Lac Ste. Marie, Ste. Agathe des Monts, Nomingue, Lac Mercier, Sherbrooke, Brome, Knowlton, Sweetsburg, Georgeville, Bolton Glen, Rigaud, Stoneham; N.B.: Waweig; N.S.: Annapolis, Baddeck; P.E.I.: Alberton; MAINE: Capens, Southwest Harbor, Casco, Orr's Island, Fort Kent; N.H.: Glen House, Randolph, Mt. Madison, Pinkham Notch, Stinson Lake, Hanover, Franconia, Bretton Woods; VT.: Plainfield, Rutland, Jacksonville, Lake Willoughby; MASS.: Dover, Amherst, Lexington, Winchendon; R.I.: Westerly, Beach Pond; CONN.: Lebanon; N.Y.: Millwood, Java, Loch Muller in Essex Co., Slide Mt. in Ulster Co., Cranberry Lake, Keene Valley, Saranac Lake, Syracuse, Lockport, Mix Creek in Cattaraugus Co., Ithaca, Bemus Point, Oneonta; PA.: Spring Brook, Dushore, Inglenook in Dauphin Co.; N.J.: Moorestown; MD.: Bowie, Chevy Chase; N.C.: Highlands at 3800 ft, Wayah Bald at 5400 ft in Macon Co.

Common as it is, this species has never been reared. It is not a meadow or grassland species. I have always taken it along forest edges or woodland paths. It occurs from the southern fringes of the Canadian Zone to the northern Carolinian Zone but in the West is confined to a narrow area along the northern edge of the Prairies. Two specimens from Robson, B.C., represent its known occurrence west of the continental divide.

***Exyston (Anecphysis) austelli* n. sp.**

This species is characterized by its uniformly upwardly curved ovipositor with uniformly tapered valvulae (Fig. 7). Within its own range it may be distinguished from *variatus* by the usually complete yellow orbits (if the orbits are incomplete the gap is colored red). *E. variatus* in the area of *austelli* always has very broadly incomplete yellow orbits. See the further remarks under *variatus*.

***Holotype***

Female; length 6 mm. Head not elongated; external apical margin of antennal scape shallowly excavated; face about twice as wide as long; cheeks in frontal aspect converging at about 65°; cheeks strongly rounded near lower margin of eye and strongly convergent behind eyes; occipital carina curving smoothly toward hypostomal carina, which it meets at an angle of about 70°, gradually becoming higher toward lower end; antenna weakly tapered toward base and apex.

Mesonotum with no lateral ridge; scutellum slightly longer than broad, strongly punctate anteriorly, highly polished, and sparsely punctate posteriorly; propodeum completely carinate, rounded in profile; apex of hind tibia with no projection.

Petiole in dorsal aspect of uniform width except for the prominent auriculae; ovipositor valves vomeriform; ovipositor evenly curved upward in the apical

third; lower valvula more or less uniformly tapered throughout its length, upper valve tapered from the base of the curve (Fig. 7).

Color of head and thorax black, yellow and red, the following parts black: frons, vertex, postocciput, central part of pronotum, central lobe of mesonotum, prescutellar scrobe, mesopleuron, mesosternum, metapleuron. Remainder of head and thorax red with the following parts yellow: mouth parts, clypeus, face, inner orbit to top of eye and circling back onto vertex, cheek to about one-half eye-height, postgena up to foramen magnum, propleuron, lower third of pronotum, prepectus and adjacent parts of mesopleuron, mesosternum centrally, tegula, subtegular ridge, scutellum, postscutellum. Abdomen red with the sides of tergites two and three black, the following tergites laterally darkened, and the following parts yellow: third and following tergites apically, the third narrowly, apical halves of fourth and fifth tergites, all visible parts of sixth and following tergites, venter and genitalia. Antennal scape red above, yellow below, pedicel and basal half of flagellum above black, remainder of antenna reddish orange to yellow. Four anterior coxae and trochanters yellow, remainder of these legs reddish except femora in front. Hind coxa red, yellow below and apically; hind trochanters yellow, remainder of hind legs reddish.

#### *Allotype*

Male; resembling the female except that the cheek is more strongly curved. Color resembling that of the holotype except in the following details: yellow inner orbit ending below top of eye, cheek not yellow above bottom of eye, outer orbit red to top of eye, remainder of temple black; propleuron and mesopleuron black; most of pronotum black, leaving only upper and lower margins red; posterior third of propodeum black; only narrow yellow apical margins present on abdominal tergites three to six.

#### *Variation*

*Female paratypes*.—Length 5–7.5 mm. Color varying from mostly red to mostly black. Black at its minimum extent occupying only the frons, ocellar triangle, extreme apex of mesonotum, pedicel, and first flagellar joint. At its maximum extent black covers, in addition to those areas listed for the holotype, the following: entire mesonotum except for notaulices, inner and outer sides of hind coxa, base and apex of hind femur. At the maximum extent of yellow, the orbits are broadly complete and the propodeum, pronotum, and hind coxa are extensively suffused with yellow.

*Male paratypes*.—Length 5–7.5 mm. Since I cannot with certainty separate males of this species from those of *variatus*, it is impossible to detail color variation. I expect it to be similar to that of female but to average less yellow and more black.

#### *Egg*

Ovate, stalk terminal, surface faintly reticulate.

*Specimens Seen* (25 ♂♂, 29 ♀♀)

*Holotype*.—Female, Highlands, 3800 ft, North Carolina, May 31, 1957, W. R. M. Mason, flying in a mass of shrubbery with specimens of *E. variatus* Prov. (CNC No. 6938).

*Allotype*.—Male, same data as holotype (CNC).

*Paratypes*.—N.C.: 5 ♂♂, ♀, same data as holotype but May 23–31 (CNC); 2 ♀♀, Whiteside mountain, 4900 ft at Highlands, June 29 and July 20, 1957, W. R. M. Mason and W. R. Richards (CNC); ♀, Wayah Bald at 4100 ft in Macon Co., July 29, 1957, W. R. Richards (CNC); ♀, Looking Glass Park in Pisgah Forest, July 19, 1957, W. R. Richards (CNC); 4 ♂♂, ♀, Hamrick, Aug. 19–29, 1950, H., M., and D. Townes (Townes); 2 ♂♂, 3 ♀♀, Crabtree Meadows at 3600 ft in Yancey Co., Aug. 5, 1951, and Aug. 21–22, 1950, Townes family (Townes). VA.: ♀, Hardscrabble Knob in Augusta Co., Aug. 31, 1934, H. A. Allard (USNM). W.VA.: ♀, Bolivar, Sept. 22, 1942, H. K. Townes (Townes). MD.: ♂, 4 ♀♀, Bowie, May 30 to June 24, 1945, H. and M. Townes (Townes); ♂, Bowie, June 21, 1946, H. H. Swift (Swift). PA.: ♀, Philadelphia, July 9, 1941, H. and M. Townes (Townes); ♂, ♀, Spring Brook, Aug. 13–25, 1944–5, H. K. Townes (Townes). N.J.: 7 ♂♂, 7 ♀♀, Moorestown. June 10 to Aug. 8, 1939, H. and M. Townes (Townes and Swift). N.Y.: ♀, New York, Sept. 1, 1946, H. H. Swift (Swift). MASS.: 3 ♂♂, 2 ♀♀, Holliston, Aug. 4 to Sept. 1, N. Banks (MCZ)).

This species occurs from Massachusetts to the Smoky Mountain area and flies continuously from May to September.

***Exyston (Anecphysis) hadros* n. sp.**

This species may be distinguished from *boreotis* by color characters discussed under that species. It may also be separated by the ovipositor, for the lower valvulae are deepest basally and gradually taper to the apex (Fig. 2). It is distinguishable from *variatus* by the cheeks, which are wider than the eyes in lateral aspect and less convergent behind the eyes in dorsal aspect. In *variatus* the cheeks are not wider than the eyes in lateral aspect and are more strongly convergent behind the eyes and more convex in dorsal aspect. In addition *hadros* is a larger and more robust species than *variatus* or *austelli* and the face is often slightly prolonged and roughened.

In general appearance some stouter and shorter specimens of *hadros* resemble *tectus* but the latter may be distinguished from *hadros* by its smaller size, shorter and more bulging face and clypeus, more strongly convergent cheeks (in frontal aspect), and more strongly convex cheeks (in dorsal aspect). *E. hadros* often has longer and proportionately slimmer abdomen, propodeum, and hind coxae than *tectus*.

***Holotype***

Female; length 8 mm; head slightly elongated, external apical margin of antennal scape shallowly excavated; face about 2.1 times as wide as long; cheeks in frontal aspect converging at about 70°; cheek decidedly convex near lower margin of eye; occipital carina gradually increasing in height

toward lower end, curving slowly toward hypostomal carina, and meeting it at an angle of about  $60^\circ$ ; antenna weakly tapered toward base and apex.

Mesonotum with no lateral ridge; scutellum longer than broad, coarsely rugulopunctate basally, densely and coarsely punctate apically; propodeum completely carinate except for central section of basal carina, rounded in profile; apex of hind tibia with no projection.

Petiole in dorsal aspect broadening throughout its length, comparatively short and broad, and stoutly built; ovipositor valves vomeriform; ovipositor sinuate, curved upward at the apical third, and decurved at the extreme apex; lower valvulae uniformly tapered, upper valvulae abruptly tapered near the apical third and thin dorsoventrally at the extreme apex (Fig. 2).

Color of head, thorax, and abdomen red with the following parts black: frons, ocellar triangle, postocciput, extreme anterior portion of mesonotum, sutures of the meso- and meta-thorax, meso- and meta-sternum, extreme base of petiolar tergite, and petiolar sternite. The following parts yellow: mouth parts, clypeus, face, inner orbit to near top of eye, cheek to one-third eye-height, postgena, propleuron, lower third of pronotum, prepectus and adjoining parts of mesopleuron, mesosternum centrally, tegula, subtegular ridge, scutellum, postscutellum, narrow apical margins of third and fourth tergites, medioapical half or more of fifth and following tergites and their lateral margins, sternites, and genitalia. Scape red above, yellow below; pedicel and extreme base of flagellum black; remainder of flagellum reddish orange. Legs red with the following yellow: four anterior coxae and trochanters, four anterior femora in front, posterior coxa below, posterior trochanters.

#### *Allotype*

Male; resembling the holotype except that the length is only 7 mm and the occipital carina is lower and meets the hypostomal carina at about  $70^\circ$ . The color is much darker than in the holotype; head and thorax black with only the following parts yellow: mouth parts, clypeus, face, extreme lower end of cheek, scutellum, postscutellum, tegula, and wing base. Abdomen black with apex of tergite two, all of tergites three and four, and most of tergite five, red; venter yellow; genitalia brown. Scape dark red above, light red below; pedicel and first flagellar joint black; remainder of flagellum reddish, darker above. Legs colored as in the holotype except the femora and coxa of middle leg completely red, hind coxa not yellow below, but black at extreme base.

#### *Egg*

Ovate; stalk apical; surface reticulate.

#### *Variation*

*Female paratypes*.—Length 5–9 mm; occipital carina meeting hypostomal carina at  $60^\circ$ – $75^\circ$ ; cheek moderately to weakly convex near bottom of eye, but always much broader than eye in lateral aspect. Color usually not varying much from that of holotype but the reddest specimens with no

black except on the pedicel and first flagellar joint. Blackest specimens with the following parts black: upper third of head, central stripe on pronotum, all except center of mesonotum; upper and lower parts of propleuron, leaving a red central stripe; most of metapleuron and all propodeum except a central transverse red stripe, basal two-thirds of petiole, basal half of tergites two, six, and seven. Extent of yellow varying little except that hind coxa is sometimes entirely red.

*Male paratypes*.—Length 7–8 mm; occipital carina occasionally weak or absent at the extreme lower end. Color often more rufous than in allotype, specimens with the largest extent of red marked almost exactly as the holotype, except for the red propleuron and black prepectus and adjoining regions.

*Specimens Seen* (14 ♂♂, 21 ♀♀)

*Holotype*.—Female, Swift Current, Saskatchewan, September 17, 1940, A. R. Brooks (CNC No. 6939).

*Allotype*.—Male, Assiniboia, Saskatchewan, June 15, 1955, A. R. Brooks (CNC).

*Paratypes*.—COLO.: ♂, Marys River, May 24, 1913, Noren (USNM); ♀, Foothills near Ft. Collins, June, 1895, C. F. Baker (USNM); ♀, Clear Creek at 6000 to 7000 ft in Jefferson Co., June 29, 1922 (USNM). ALTA.: ♀, Eisenhower Junction in Banff National Park at 4700 ft, July 19, 1955, R. Coyles (CNC); ♀, Banff, July 1, 1922, C. B. D. Garrett (CNC). SASK.: ♀, Cut Knife, June 7, 1940, A. R. Brooks (CNC); 2 ♀♀, Prince Albert National Park, July 19, 1941, Aug. 15, 1940, J. G. Rempel (Townes); 2 ♂♂, 2 ♀♀, Regina, June 14 to Aug. 13, 1940, and 1944, J. G. Rempel (Townes); ♂, 2 ♀♀, Qu'Appelle, June 28, 1944, J. G. Rempel (Townes). MINN.: 2 ♂♂, Itasca Park, July 2, 1937, A. E. Pritchard, and Sept. 5, 1913, S. A. Graham (Minn.); ♀, Loman, June 8, 1934, D. G. Dening (Minn.); ♀, Mille Lacs Co., June 3, 1939, H. E. Milliron (Minn.). MICH.: ♀, George Reserve in Livingstone Co., Sept. 2, 1940, Geo. Steyskal (Dreisbach); ♀, Trout Lake in Cheboygan Co., Aug. 25, 1925, H. B. Hungerford (Kansas).

This species has a range mainly in the Transition Zone from Alberta to Colorado and Michigan. Females appear to fly throughout the summer but captures of males in May, June, and September indicate a double generation.

***Exyston (Anecphysis) boreotis* Davis**

*Exyston boreotis* Davis, 1897, Trans. Am. Entomol. Soc. 24, 238 (o.d., ♀, Hudson Bay Territory, type in ANSP).

*Exyston variatus* Mason, 1951, in Hymenoptera of America North of Mexico Synoptic Catalogue. Edited by Muesebeck, Krombein, and Townes. U.S. Dept. Agr., Agr. Monograph, No. 2, p. 231 (in part).

The type probably came from Alberta or Saskatchewan, because these were part of the Hudson Bay Territory in the 19th Century.

This species is characterized by the sinuate ovipositor (Fig. 6). The lower valvulae are deepest just beyond the middle and taper rapidly to the

apex; the upper valvulae are strongly depressed at the extreme apex, appearing very shallow apically in lateral view.

The females may be distinguished from all other species of the *variatus* group by the black postgena and hind coxa and from *austelli* and *hadros* by the black thorax. The cheeks are only weakly convex, being intermediate in shape between typical species of the *variatus* and *speciosus* groups. This species also bears considerable resemblance to *E. ater* but differs in its convex cheeks and completely black postgena (some specimens of *ater* from the Pacific coast have completely black postgena but these are small, slim individuals with no red markings on the abdomen).

#### *Holotype*

This is a redescription of Davis's type specimen.

Female; length 8 mm; head somewhat elongated; external apical margin of antennal scape shallowly excavated; face about twice as wide as long, cheeks in frontal aspect converging at about 70°; cheek decidedly convex near bottom of eye although not strongly bulging, but making an abrupt angle of over 120° with postgena; occipital carina situated at outer corner of this angle, not strongly elevated, but curving abruptly towards hypostomal carina and meeting it at an angle of about 80°; of uniform height throughout its length.

Mesonotum without any lateral ridge; scutellum rugulopunctate anteriorly, but shallowly punctate posteriorly; propodeum completely carinate and rounded in profile; apex of hind tibia with no projection.

Sides of petiole in dorsal aspect subparallel; abdomen rather large and heavy set; ovipositor valves vomeriform; ovipositor straight and sharply tipped but sinuate apically; curved upward at apical 0.25, and weakly curved downward at extreme apex; upper valvulae strongly depressed at apical 0.05; lower valvulae deepest at middle (Fig. 6).

Color of head, thorax, and abdomen black; red limited to the narrow apical margin of tergite two and most of tergite three, except the lateral margin centrally; the following parts yellow: mouth parts, clypeus, face, inner orbit halfway to top of eye, cheek from lower quarter of eye to junction of hypostomal and mandibular carinae, propleuron except for lateral black marks, lower margin of pronotum, tegula, subtegular ridge, wing base, spot about sternaulus, margin of prepectus below sternaulus, narrow margins of tergites three and four, apical half and lateral margins of tergites five and six, all visible parts of tergite seven. Antennal scape and pedicel black, flagellum black basally, yellowish orange below and apically; scape yellow below. Four anterior legs red, with yellow coxae and trochanters; hind coxa entirely black; hind trochanter yellow; hind femur red with narrow basal, and apical black annuli; hind tibia and tarsus dark brown.

#### *Egg*

Ovate, stalk terminal, surface unsculptured (Fig. 6).

### Male

No males have been definitely associated with the females of this species. A few males which I have tentatively placed here agree morphologically with the females, especially in the rounded cheeks, and have an essentially similar color pattern, with the normal sexual reduction in extent of yellow.

### Variation

*Females*.—Length 6.5–9.5 mm; body often rather long and slim. Color varying little from that of holotype, except that abdomen is often much more extensively red; apex of first tergite, all of second and third, and all but subapical band on fourth tergite, frequently red, and sometimes central part of fifth tergite red also. One specimen from Colorado has red propodeum and hind coxa but is otherwise typically colored. Antennal scape usually black; yellow margins of pronotum, prepectus, and mesopleuron reduced or sometimes absent. Egg sometimes with faint reticulations near attachment of stalk.

### Specimens Seen (18 ♂♂, 10 ♀♀)

*Specimens*.—COLO.: 7 ♂♂, near Estes Park, June 13–15, 1948, Townes family (Townes); ♀, Elk River in Jefferson Co., July 1894, C. F. Baker (USNM); ♀, Fort Collins, C. F. Baker (USNM). WYO.: ♀, Mammoth Hot Springs in Yellowstone National Park, C. T. Brues (MCZ). ALTA.: 2 ♂♂, Grimshaw, June 18, 1939, A. W. E. Erikson (Alta.); ♂, Edmonton, June 24, 1945, E. H. Strickland (Townes); ♂, Bilby, July 12, 1924, O. Bryant (Alta.); ♂, ♀, Nordegg, July 21, 1926, E. H. Strickland (CNC); 2 ♂♂, Banff, June 22 and July 1, 1922, C. B. D. Garrett (CNC); ♀, Banff, June 13, 1928, O. Bryant (USNM); ♀, Radnor near Cochrane, June 25, 1932, O. Peck (CNC). SASK.: ♀, Regina, June 14, 1940, J. G. Rempel (Townes); 2 ♂♂, ♀, Waskesiu, June 9–19, 1938, J. G. Rempel (Townes). H.B.T.: ♀, no further data but probably meaning part of modern Alberta or Saskatchewan (type) (ANSP). MICH.: ♀, Saginaw Co., June 1, 1940, R. R. Dreisbach (Dreisbach); ♂, no further data (USNM). ?N.Y.: ♂, Mud Creek in Tomkins Co., June 17–20, 1904 (CU).

This species occurs in the Transition and Canadian zones from Michigan to the Continental Divide but seems to be especially characteristic of the parkland belt of the Canadian Prairies. It flies in June and early July.

### SPECIOSUS GROUP

This group includes the Nearctic species *speciosus*, *ater*, *californicus*, *reniformis*, *lophotos*, and the Holarctic *spinulosus*.

The group is distinguished by the cheek, which is flat, especially in the female, at the level of the lower margin of the eye. In addition the occipital carina often conspicuously projects outward at right angles to the cheek (Fig. 16). The cheek makes a sharp angle with the postgena, and the occipital carina lies at this angle. Species of this group may be distinguished from the *marginatus* group by the characters in Table II.

***Exyston (Ancephysis) speciosus* Davis**

*Exyston speciosus* Davis, 1897, Trans. Am. Entomol. Soc. 24, 237 (o.d., ♂, Colo., type in ANSP).

This species is characterized in both sexes by the absence of the occipital carina from below the level of the middle of the eyes; in other respects it resembles other species of the *speciosus* group.

***Female***

Length 5 to 8.5 mm; head not elongated; external apical margin of antennal scape shallowly excavated; face about 2.4 times as wide as long; cheeks in frontal aspect converging at about 80°; cheek flat near lower margin of eye but curving smoothly onto the postgena, occipital carina absent from below level of middle of eye, but extreme lower end of occipital carina sometimes indicated weakly as a small ridge subtending the hypostomal carina; cheek at an angle of about 110° with the postgena; antenna weakly tapered toward base and apex.

Mesonotum with no lateral ridge; scutellum slightly longer than broad, more or less rugulose anteriorly, highly polished and strongly punctate posteriorly; propodeum completely carinate, rounded in profile; apex of hind tibia with no projection.

Petiole in dorsal aspect slightly broadening throughout its length; ovipositor valves vomeriform; ovipositor weakly sinuate and sharply pointed, slightly decurved at extreme apex (Fig. 4).

Color of head and thorax black, the following parts yellow: mouth parts, clypeus, inner orbit usually to near top of eye, cheek to one-third eye-height, postgena to foramen magnum, propleuron, tegula, wing base, subtegular ridge, prepectus and adjacent parts of mesosternum and mesopleuron, mesosternum centrally, apical half, or all, scutellum, postscutellum. Abdomen mostly red to mostly black, with the following parts yellow: very narrow apical margin of third tergite, narrow apical and lateral margins of fourth tergite, broad apical and lateral margins of fifth tergite, apical half or more of sixth and following tergites, genitalia, and venter except for petiolar sternite. In one specimen from southern Utah yellow markings on abdomen are much more extensive. At its minimum extent red is confined to apex of second tergite and central parts of third and fourth: black at its minimum extent is confined to basal two-thirds of petiole. Antenna reddish orange, black basally and above, the scape sometimes yellow below. Four anterior coxae and trochanters yellow; four anterior legs red, usually yellow anteriorly; hind coxa black or red, but yellow below; hind trochanter yellow; hind femur red, usually with basal and apical black annuli; hind tibia red to brown with poorly defined apical dark annuli, occasionally yellow externally; hind tarsus brown; wings infumated.

***Male***

Resembling the female except that the occipital carina sometimes ends as low as the lower margin of the eye, and the cheek is sometimes convex.

The color generally is darker than in the female, the yellow pattern differing as follows: yellow inner orbit seldom extending above antennal socket; cheek and postgena entirely black; thorax entirely black except for tegula, apex of scutellum and, sometimes, postscutellum; abdomen yellow only on narrow apical margins of fourth to sixth tergites and basal sternites. Abdomen much more extensively black, in the extreme rufous only tergites two to four red; in the extreme melanic only a central castaneus area on tergite three remaining reddish, but apical margins of second and third tergites pale brown. Four anterior coxae black to red basally; middle femur sometimes black basally and internally; hind coxa completely black; hind femur sometimes black. Antenna black, dark to light brown below and apically.

#### *Egg*

Ovate; surface usually reticulate; stalk apical (Fig. 4).

*Specimens Seen* (16 ♂♂, 24 ♀♀)

*Holotype*.—Male, Colorado (ANSP).

*Other specimens*.—B.C.: Diamond Head Trail near Squamish at 3300 ft, Kamloops, Nickel Plate Mine near Hedley at 5000 ft, Robson. WASH.: Elbe, Big Four Mountain, Mt. Rainier at 4700 to 5500 ft. OREG.: Cannon Beach, Seaside, Lick Creek Ranger Station, Diamond Lake at 5162 ft, head of Blitzen River in Steens Mountains at 7000 ft, Wallowa Lake at 6500 to 7000 ft. CALIF.: Meadow Valley in Plumas Co. at 3500 to 4000 ft, Echo Lake at 7800 ft in Eldorado Co. ALTA.: Banff. IDAHO: Headquarters, Chatcolet. MONT.: Summit Station at 5500 ft. WYO.: Swan Lake in Yellowstone Park, Big Horn Mountains near Buffalo at 6000 ft. UTAH: Navaho Lake in Dixie National Forest.

This species flies in midsummer, males having been taken between June (California) and early August (coastal Oregon and British Columbia). It apparently flies somewhat earlier in interior localities, most of the captures from the Rocky Mountain region being in June and July.

#### *Exyston (Anecphysis) spinulosus* n. sp.

*Exyston contractus* Davis, Mason, 1951, in *Hymenoptera of America North of Mexico*, Synoptic Catalogue. Edited by Muesebeck, Krombein, and Townes. U.S. Dept. Agr., Agr. Monograph, No. 1, p. 231 (in part, misdet.).

This species is distinguished by the ovipositor, which is comparatively broad at the tip and strongly decurved, the dorsal edge bearing several conspicuous sharp spines (Fig. 5). The egg has a terminal stalk, is about 2.5 times as long as wide, and is covered with a reticulate pattern (Fig. 5). The color pattern of the female is not distinctive, being the same as that of *reniformis* and *californicus*, but it may be distinguished from *lophotos* and *boreotis* by the yellow mesosternum, bicolored hind coxa, and yellow postgena. The male may be distinguished only by association with the female.

*Holotype*

Female; length 8.5 mm; head somewhat elongated (Fig. 17); externoapical margin of antennal scape broadly excavated; face about twice as wide as long; cheeks in frontal aspect converging at about  $60^\circ$ ; cheek flat near bottom of eye, making an abrupt angle of about  $110^\circ$  with postgena (Fig. 18); occipital carina situated at outer corner of this angle, curving abruptly toward hypostomal carina, meeting it at an angle of about  $70^\circ$ , and becoming much weaker at its lower end.

Mesonotum without any lateral ridge; propodeum completely carinate, rounded in profile; apex of hind tibia with no projection.

Petiole broadening throughout its length; ovipositor valves vomeriform; ovipositor straight but strongly decurved at extreme apex and rather broad with several small dorsoapical median spines.

Color of head and thorax black, the following parts yellow: mouth parts, clypeus, face, inner orbits above antennal sockets, cheeks to one-third eye-height, postgena, propleuron, lower margin of pronotum, tegula, wing base, subtegular ridge, apical two-thirds of scutellum, postscutellum, prepectus, mesosternum centrally, and adjacent parts of mesopleuron. Four anterior coxae and trochanter yellow, remainder of front and middle legs red, but anterior femur yellow in front. Hind coxa black above and basally, red laterally, ventrally, and apically; hind trochanters yellow; hind femur red; hind tibia brown with a subbasal black annulus, and a median incomplete yellowish annulus; hind tarsus brown. Antenna reddish orange; darker dorsally, and black basally; scape reddish below. Abdomen red; narrow apical margins of second and third tergites yellow; broad apicomedian margins of fifth and following tergites, and their lateral margins, yellow; petiole and extreme lateral margins of second, third, and fourth tergites black; remainder of fifth, sixth, and seventh tergites black; venter and genitalia yellow.

*Allotype*

Male; resembling the type except in the following details: hypostomal carina of uniform height, cheek slightly convex, face centrally rather coarsely rugulopunctate.

Color resembling that of the female except for the following reductions of yellow: upper part of face black, postgena and hind part of cheek black, thorax completely black, tegula brown, wing base yellow, anterior coxa black basally, middle and hind coxae completely black. Yellow of abdomen confined to narrow apical margins of fifth and sixth tergites, and basal sternites. Hind femur red, suffused with black internally and at base and apex; hind tibia uniformly brown. Scape with only a small yellow spot below. Abdomen entirely black except for yellow areas mentioned above and the following red areas: apex of second tergite, third tergite except apicolateral quarters.

*Egg*

Long-ovate, about 2.5 times as long as broad; surface reticulate; stalk terminal (Fig. 5).

### Variation

*Female paratypes*.—Length 5.5–8.5 mm; occipital carina varying in height, sometimes of equal strength throughout its length. Extent of yellow markings least in northern specimens and greatest in southern ones. In northernmost specimens outer part of postgena black, yellow of prepectus and mesosternum reduced to a narrow line along prepectus behind fore coxae, hind coxa entirely black, basal half of second tergite black, broad lateral margins of succeeding tergites black, and scape entirely black. Minimum extent of black in specimens from the Mississippi Valley as follows: inner orbit broadly to top of eye, gena and postgena to foramen magnum, lower third of pronotum, lower anterior third of metapleuron, apicoventral two-thirds of hind coxa, posterior edge of hind tibia and broad central annuli, lower half of scape; second to fourth abdominal tergites sometimes entirely red except for yellow medio-apical margin of third tergite. Hind coxa often red above.

*Male paratypes*.—Length 5–9 mm, cheek varying from flat to weakly convex near lower margin of eye; proportions of petiole and hind legs varying, both sometimes quite short and broad. Black color more extensive in northern specimens and yellow color more extensive in southern specimens, the blackest specimens as follows: large area in center of face black; red areas of femora replaced by black; red areas of abdomen replaced by black except apical margin of second tergite, and vague central suffusion on third tergite. In southern specimens the yellow areas have greater extent as follows: scape below, face completely, extreme lower end of cheek, apical half of scutellum, postscutellum, front coxa entirely, middle coxa apically, broad central annulus on hind tibia, narrow apical margins of second and third tergites, broad to very broad, apical margins of fourth and following tergites, venter, and genitalia. At its greatest extent red covers all second to fourth tergites except dark lateral, and yellow apical, margins, and all of hind femur except narrow, poorly defined, apical annuli.

*Specimens Seen* (32 ♂♂, 40 ♀♀)

*Holotype*.—Female, Norman Wells, Northwest Territories, July 3, 1949, W. R. M. Mason, flying over open weedy bank of Mackenzie River (CNC No. 6941).

*Allotype*.—Male, same data as holotype except June 29 (CNC).

*Paratypes*.—U.S.S.R.: Kamchatka; ALASKA: Fairbanks; Y.T.: Rampart House, Firth River near arctic coast; WASH.: Mt. Adams; WYO.: Buffalo in Big Horn Mountains, Grand Teton National Park; SASK.: Attons Lake near Cut Knife, Indian Head; N. DAK.: Fargo, Mott, Minot, Tower City; S. DAK.: Harrold; MINN.: St. Paul, Mentorville, Crookston, Pine River, Kittson Co., Marshall Co., Traverse Co., Polk Co., Hennepin Co., Houston Co.; IOWA: Henry Co., Dickinson Co.; KANS.: Saline River at Hays; MO.: Leeton.

This species has a very wide range from the Hudsonian to the Upper Austral zones and from Kamchatka to the upper Mississippi Valley but apparently is rare in the mountain regions of the western U.S.A.

A short flight in early summer is indicated, since all specimens, except at the most northern localities, were taken in June.

***Exyston (Anecphysis) reniformis* n. sp.**

This species is distinguished by the shape of the egg. This is reniform with the stalk centrally attached and bears no external flanges (Fig. 8). The female may be distinguished from that of *boreotis* by the yellow postgena and from *lophotos* by the centrally yellow mesosternum. It cannot be distinguished from *californicus* or *spinulosus* except by characters of the egg and ovipositor. Males from the type locality in Colorado may be distinguished from most other males in this group by the hind coxae, which are yellow or reddish apically below, the completely yellow face, and truncated inner orbits, which are yellow to well above the antennal sockets. Californian males of *californicus* cannot be distinguished from this species by their color pattern but males of *californicus* from Colorado have completely black hind coxae. A male of this species from Penticton, B.C., fails to exhibit the above-mentioned distinguishing color pattern.

*Holotype*

Female; length 8 mm; head somewhat elongated; externoapical margin of antennal scape broadly excavated; face about twice as wide as long; cheeks in frontal aspect converging at about 65°; cheek flat near bottom of eye, making an abrupt angle of about 100° with postgena; occipital carina situated at outer corner of this angle, extending outward in a plane about parallel with postgena, curving abruptly towards hypostomal carina and meeting it at an angle of about 80°, tall and strong throughout its length.

Mesonotum without any lateral ridge; propodeum completely carinate, in profile rounded and not strongly declivous; apex of hind tibia with no small projection.

Petiole in dorsal aspect slightly broadening throughout its length; ovipositor valves vomeriform; ovipositor straight, moderately sharply pointed, the extreme apex decurved (Fig. 8).

Color of head and thorax black, the following parts yellow: mouth parts, clypeus, face, inner orbits nearly to top of eye, gena and postgena to one-third eye-height and to foramen magnum, propleuron, lower margin of pronotum, prepectus, mesosternum centrally, small areas adjoining prepectal carina immediately above and below sternalix, subtegular ridge, tegula, wing base, apical half of scutellum, postscutellum. Four anterior legs yellow; tibiae and femora reddish behind; tarsi light brown; hind coxa black, yellow below and apically; hind trochanter yellow; hind femur red with basal and subapical dark annuli and extreme apex yellow; hind tibia brown with a subbasal blackish annulus; hind tarsus brown. Abdomen red with basal three-quarters of petiole black, and sides of fifth and sixth tergites black basally, the following

parts yellow: small median apical marks on first three tergites; large indefinite apical medial areas on fourth and following tergites, the sixth and seventh being almost entirely yellow, venter and genitalia and lateral margins of fifth and following tergites. Antenna brownish orange, black basally and above; scape yellow below.

#### *Allotype*

Male; resembling the female except for cheek being weakly convex near lower margin of eye, and hypostomal carina being only about half as tall.

Color like that of female but with the following reductions of yellow areas: postgena and gena completely black, clypeal suture outlined in black, face centrally with three incomplete vertical black lines, inner orbits black above antennal sockets, thorax and propodeum completely black except extreme tip of scutellum, tegula, and wing base yellow. Four anterior coxae black basally, anterior and middle femora black to red behind; hind coxa completely black; hind femur black, but reddish above and extreme apex yellow; hind tibia brown with a broad incomplete yellow annulus; hind tarsus brown. Abdomen black except for central 0.8 of second and third tergites and central half of fourth tergite; apical yellow margins of tergites narrow and confined to tergites three to six; venter of abdomen yellow. Antenna black, brown below and apically; scape yellow below.

#### *Egg*

Reniform-ovate; surface reticulate but with no flange; stalk centrally attached and very short; anchor button-like (Fig. 8).

#### *Variation*

*Female paratypes*.—Length 4.5–8 mm; cheek near lower margin of eye occasionally very weakly convex; hypostomal carina sometimes weakly developed but of uniform height throughout its length; costula often weak or absent. Color of head and thorax showing very little variation from that of type except for Californian specimens being suffused with red on mesonotum, mesopleuron, propleuron, and hind coxae. Black color of abdomen may be restricted to base of petiole or may cover all of petiole, lateral margins of second, third, and fourth tergites and basal half of fifth and following tergites. Apical black annulus of hind femur often reduced or absent.

*Male paratypes*.—Center of face varying from entirely yellow to black; hind femur varying from entirely black to mostly red, with broad basal and narrower apical black annuli. In specimens with reddest abdomens, second tergite like that of allotype, third and fourth tergites entirely red: in darkest abdomens, red confined to third, apex of second, and base of fourth tergites.

*Specimens Seen* (6 ♂♂, 26 ♀♀)

*Holotype*.—Female, near Estes Park, Colorado, June 15, 1948, Townes family (Townes).

*Allotypes*.—Male, same data as holotype (Townes).

*Paratypes*.—COLO.: 3 ♂♂, 11 ♀♀, same data as holotype but June 12 to 15 (Townes); ♀, Boulder, June 24, 1933, M. T. James (Townes); 5 ♀♀, Steamboat Springs, Aug. 5-6, 1948, Townes family (Townes); ♂, Steamboat Springs, July, 1894, C. F. Baker (USNM). CALIF.: ♀, Cedar Pass in Modoc Co., July 8, 1946, P. D. Hurd (Calif.); ♀, Viola in Shasta Co., June 27, 1947, T. F. Leigh (Calif.). OREG.: ♀, 5 miles west of Suttle Lake, July 8, 1939, Grey and Schuh (Oreg.). B.C.: ♂, 2 ♀♀, Shingle Creek near Penticton at 2000 ft, May 23, 1953, J. R. McGillis (CNC); ♀, Robson, June 29, 1947, H. R. Foxlee (CNC); ♀, Okanagan, August (CNC); ♀, Fort Nelson, June 13, 1948, W. R. M. Mason (CNC). ALTA.: ♀, Eisenhower Junction at 4700 ft, in Banff National Park, July 6, 1955, J. R. McGillis (CNC).

The species is widespread in the Transition and lower Canadian zones of the western mountains. Males emerge in May and June, but females are on the wing until after midsummer.

***Exyston (Anecphysis) lophotos* n. sp.**

This species is characterized by the reniform egg with a short central stalk and a large posterior sagittal flange (Fig. 9). The female may be distinguished from other members of the *speciosus* group by having the yellow of the mesopleuron and mesosternum confined to a narrow band along the prepectal carina and by the frequent extensive suffusion of red on the thorax. In addition the hind coxa is usually mostly red, and never yellow below.

*Holotype*

Female; length 8.5 mm; external apical margin of antennal scape broadly excavated; face about twice as wide as long, cheeks in frontal aspect converging at about 60°; cheek flat behind lower part of eye, making an abrupt angle of about 105° with postgena; occipital carina situated at the corner of this angle and extending strongly outwards in plane of postgena; carina curving towards the hypostomal carina and of uniform height, joining it at an angle of about 70°.

Mesonotum without a lateral ridge; scutellum a little longer than broad, coarsely rugosopunctate anteriorly, but shining and finely punctate posteriorly; propodeum completely and strongly carinate, rounded in profile; apex of hind tibia without a projection.

Petiole in dorsal aspect broadening uniformly throughout its length; ovipositor valves vomeriform; ovipositor very slightly curved upward and nearly uniformly tapered to a sharp apex which is broader than deep (Fig. 9).

Head and thorax black, the following parts yellow: mouth parts, clypeus, face, inner orbit to slightly above antennal socket, cheek to one-third eye-height, postgena to foramen magnum, propleuron, lower margin of pronotum, a narrow broken line along prepectal carina, scutellum, postscutellum, subtegular ridge, tegula, and wing base. Propodeum red; sides of pronotum suffused with red. Antenna reddish orange, black basally; scape red below. Four anterior legs red, the following parts yellow: anterior coxa, middle coxa apically, front of anterior femur, apex of both femora, trochanters.

Hind leg red, the trochanter yellow, the hind femur and tibia both with a poorly indicated dark basal annulus, tarsus darkened. Abdomen red; petiole darkened basally; apical margins of third and following segments narrowly yellow, apical margins of fifth and following segments very broadly yellow, genitalia and subgenital plate yellow.

*Allotype*

Male; resembling the female in morphology.

Color like that of female but with the usual lesser extent of yellow markings. Head and thorax completely black, except for the following yellow areas: mouth parts, clypeus, triangular orbital marking above clypeus, tip of scutellum. Scape completely black; anterior and middle coxae black basally, hind coxae completely black, petiole black, sixth and seventh tergite black with only narrow apical margins on third and following tergites, second and fifth tergites with basal black infusions, propodeum black.

*Egg*

Reniform-ovate, the surface reticulate; bearing a large, posterior, sagittal flange; stalk very short and broad, elongated longitudinally (Fig. 9).

*Variation*

*Females*.—Length 7–8.5 mm. Smaller specimens are decidedly slimmer than the type. Ground color of thorax varying from entirely black to black very extensively suffused with red on mesopleuron and mesoscutum as well as propodeum and pronotum. Hind coxa varying from entirely red to entirely black, often basal half or more of petiole black. In the darkest abdomen (Forks, Wash.), the following parts are black: petiole, base of second tergite, fourth and following tergites basally. Scape usually completely black; hind femur sometimes without an apical dark annulus; hind tibia and tarsus often brown.

*Males*.—Length 5–8 mm. Face varying from almost completely black to almost completely yellow with only the upper central part black; front and middle femora sometimes black behind, hind femur, tibia, and tarsus varying from red to black; abdomen varying from red, as in allotype, to completely black except for reddish apical margins of the second tergite, the yellow markings remaining unchanged. These melanic specimens occur on the Pacific Coast and are much smaller and slimmer than the allotype.

*Specimens Seen* (6 ♂♂, 11 ♀♀)

*Holotype*.—Female, Roche Percee, Saskatchewan, July 4–8, 1927, E. and S. Criddle (CNC No. 6942).

*Allotype*.—Male, same data as holotype (CNC).

*Paratypes*.—CALIF.: ♂, ♀, Crescent City, Aug. 2, 1940, H. and M. Townes (Townes). WASH.: 3 ♂♂, ♀, Forks in Clallam Co., July 4, 1920, E. P. Van Duzee (Calif.); ♂, Port Angeles, July 16, 1945, swept from *Lathyrus torreyi*, R. D. Shenefelt (Wisc.); ♀, Tacoma, June 23, 1946, swept from *Lathyrus* spp., C. Johansen (Wisc.). B.C.: ♀, Kamloops, June 1, 1938,

G. S. Walley (CNC); ♀, Fernie, Aug. 16, 1935, Hugh Leech (USNM); ♀, Midday Valley at Merritt, July 13, 1923, R. Hopping. N.W.T.: ♀, Norman Wells, July 13, 1949, swept from vetch, W. R. M. Mason (CNC). ALTA.: ♀, Edmonton, July 3, 1932, O. Peck (CNC); ♀, Waterton, July 4, 1924, H. L. Seamans (CNC). SASK.: ♀, Willowbunch, July 28, 1955, C. D. F. Miller (CNC).

This species occurs in the Canadian and Transition zones of western Canada and extends down the Pacific Coast of the U.S.A.

The flight period is in midsummer, and the fact that several specimens are associated with legumes suggests a host feeding on this family.

***Exyston (Anecphysis) californicus* n. sp.**

This species may be distinguished from others of the group by the ovipositor, which is strongly curved upward in the apical quarter (Fig. 10), and the egg, which has a terminal stalk, a reticulate surface, and is only about one and a half times as long as wide (Fig. 10). Characteristic coloring in the female includes postgena and mesosternum yellow, hind coxae red above and yellow below, and the generally large extent of red on abdomen. Males from California are characterized by having face yellow, inner orbit yellow above antennal socket, and hind coxa yellow below, but males from Colorado or farther north are not thus characterized.

*Holotype*

Female; length 6.5 mm; head somewhat elongated; external apical margin of antennal scape broadly excavated; face about twice as long as wide; cheeks in frontal aspect converging at about 65°; cheek flat near bottom of eye, making an abrupt angle of about 100° with postgena; occipital carina situated at outer corner of this angle, strongly developed and of uniform height, lying in a plane parallel to the postgena, meeting hypostomal carina at about 70°.

Mesonotum without any lateral ridge; scutellum coarsely punctate, posteriorly, rugulopunctate anteriorly; propodeum completely carinate, rounded in profile; apex of hind tibia with no projection.

Petiole in dorsal aspect slightly broadening throughout its length; ovipositor valves vomeriform; ovipositor straight in basal three-quarters, but curved upward in apical quarter and ending in a sharp point (Fig. 10).

Color of head and thorax black, the following parts yellow: mouth parts, clypeus, face, inner orbit to near top of eye, cheek to one-third eye-height, postgena to foramen magnum, propleuron, lower third of pronotum, prepectus, mesosternum centrally, parts of mesopleuron adjoining prepectus, subtegular ridge, tegula, wing base, scutellum, postscutellum. Sides of propodeum with small red infusions. Four anterior coxae and trochanters yellow, anterior legs red, femur yellow in front, middle leg red, femur yellow in front. Hind coxa red, yellow ventrally and apically, extreme base black; hind trochanter yellow; hind femur red; hind tibia and tarsus reddish brown. Abdomen red,

basal half of petiole infused with black; apical margins of third and fourth tergites narrowly yellow, apical and lateral margins of following tergites very broadly yellow; venter and genitalia yellow. Antenna reddish brown, darker above, black basally; scape yellow below.

#### *Allotype*

Male; resembling the female except in the following details: length 7 mm, scutellum much more densely punctate.

Color resembling that of female except for the usual reduction of yellow. Inner orbit yellow only slightly above antennal socket; only lower portion of cheek yellow and postgena black; propleuron black; only lower corner of pronotum yellow; mesosternum and prepectus black; mesopleuron black except for subtegular ridge; hind coxa black, yellow below and apically. Petiole black except small red apical mark; apical margin of fifth tergite only narrowly yellow. Sixth and seventh tergites black. Sixth tergite with broad median apical yellow margin, sixth to eighth sternites dark brown. Antenna dark brown, paler apically below and basally black; scape yellow below.

#### *Egg*

Short ovate, only about 1.5 times as long as wide, surface reticulate; stalk apical (Fig. 10).

#### *Variation*

*Female paratypes*.—Length 5–9 mm. Cheek near lower margin of eye sometimes weakly convex. Head and thorax with the black coloration often infused by red in Californian specimens; in the extreme rufous variants black confined to mesosternum, sutures of thorax, frons, vertex, and post-occiput. Extent of yellow markings varying but little, chief variation being in the pronotum, from lower margin yellow to lower half yellow and the hind coxa from lower apical two-thirds to a ventral line only, ground color of abdomen varying from completely red, as in many Californian specimens, to red with the following parts black: petiole, all of second tergite, apical and broad lateral margins of third and fourth tergites, basal parts of fifth and following tergites. Hind coxa varying from black to red, but always yellow below, and usually yellow apically; hind femur often with basal and apical black annuli; hind tibia usually with a subbasal black annulus.

*Male paratypes*.—Length 5–8.5 mm, cheek varying from flat to moderately convex. Extent of yellow markings varying from that of type, which is near maximum, to a minimum as follows: face centrally, and scape below, black; pronotum, prepectus, mesopleuron, and mesoscutum completely black, hind coxa basally, to almost completely, black; middle coxa with only an apicoventral yellowish mark or completely black. Red color of abdomen varying from that of type, which is nearly the maximum, to a minimum of red as follows: apical margin of second tergite, and third and fourth tergites centrally and basally. Front and middle femora sometimes darkened basally

on inner side, hind femora varying from entirely red to black with central rufous infusion on outer side.

*Specimens Seen* (about 160 ♂♂ and ♀♀)

*Holotype*.—Female, Fish Camp, California, July 15, 1948, Townes family (Townes).

*Allotype*.—Male, same data as holotype (Townes).

*Paratypes*.—CALIF.: about 90 ♂♂ and ♀♀, same data as holotype but July 11 to 14 (Townes); about 30 ♂♂ and ♀♀, Crane Flat in Yosemite Park, July 22 to 25, 1948, Townes family (Townes); 3 ♂♂, ♀, near Glacier Point in Yosemite Park, July 18 to 20, 1948, Townes family (Townes); ♀, Graegle, June 17, 1949, E. Schlinger (Townes); ♀, Mammoth Lakes in Mono Co., July 29, 1940, O. E. Hardy (Kans.); ♀, Baxter in Nevada Co., Aug. 25, 1948, P. D. Hurd (Calif.); ♀, Serra Meadow in Grant Forest, July 1, 1928, E. A. McGregor (USNM); ♀, Giant Forest in Tulare Co., July 18, 1923, C. L. Fox (Calif.); 2 ♀♀, Sequoia National Park, July 29, 1940, Kuitert and Lipovsky (Kans.); ♀, Big Bear Lake in San Bernardino Mountains, July 16, 1934, E. G. Anderson (USNM); ♂, 2 ♀♀, Bluff Lake in San Bernardino Co., July 15, 1934, C. D. Mitchener (Townes); ♀, Crystal Lake in Los Angeles Co., June 29, 1950, T. R. Haig (Calif.). COLO.: 5 ♂♂, 6 ♀♀, Rabbit Ears Pass at 9500 ft, Aug. 7, 1948, Townes family (Townes); 2 ♂♂, ♀, Rabbit Ears Pass, July 21, 1896, C. F. Baker (USNM). WYO.: ♀, Shoshone Canyon in Big Horn Mountains at 6500 ft, June 29, 1940, H. and M. Townes (Townes); ♀, northwest entrance of Yellowstone Park, July 27, 1923, A. L. Melander (MCZ). MONT.: ♀, Beaver Creek in Gallatin Co. at 6300 ft, Aug., 1913, S. J. Hunter (Kans.). ALTA.: ♀, Cowley, June 29, 1918, R. N. Chrystal (CNC). IDAHO: ♀, Stanley at 6200 ft, July 10, 1926, R. W. Haegele (USNM). OREG.: ♀, Powder River 26 miles east of Baker at 3000 ft, Aug. 9, 1937, Bolinger and Jewett (Oreg.). ALASKA: ♂, 4 ♀♀, King Salmon, Naknek River, July 23 to Aug. 4, 1952, W. R. M. Mason (CNC).

This species is common in moist meadows in the Canadian and Upper Transition zones of the mountains of the western United States. In a similar habitat in the Hudsonian Zone of southwestern Alaska I have taken a series of specimens closely matching those from Colorado. This wide gap is most probably only an apparent one due to faulty collecting.

The adults emerge in late June to mid-July throughout the range and females fly until late August.

***Exyston (Anecphysis) ater* n. sp.**

This species may be distinguished from others of the *speciosus* group except *speciosus* by the egg, which is apically stalked and has no surface reticulation, and by the sinuate ovipositor, which ends in a thin point, is weakly curved upward in the apical quarter and weakly decurved at the extreme apex (Fig. 3). The female is characterized by the black postgena and reduction of yellow on the mesopleuron, mesosternum, and prepectus. *E. lophotos*

often has the postgena partly black but the black coloration is adjacent to the hypostomal carina and does not cover the entire postgena, whereas in *ater* the black color either covers the entire postgena, or the part adjacent to the hypostomal carina is narrowly yellow.

#### *Holotype*

Female; length 6 mm; head somewhat elongated; external apical margin of antennal scape shallowly excavated; face about twice as wide as long; cheeks in frontal aspect converging at about 70°; cheek flat near bottom of eye, making an abrupt angle of about 120° with postgena; occipital carina situated at outer corner of this angle, not strongly elevated but curving abruptly towards hypostomal carina and meeting it at an angle of about 80°, of uniform height throughout its length.

Mesonotum without any lateral ridge; scutellum rugulopunctate anteriorly but shallowly punctate posteriorly; propodeum completely carinate and rounded in profile. Apex of hind tibia with no projection.

Sides of petiole in dorsal aspect parallel; the abdomen unusually long and slim, especially the second tergite. Ovipositor valves vomeriform; ovipositor straight and sharply tipped; weakly sinuate apically, curved upward at apical 0.25 and weakly decurved at extreme apex (Fig. 3).

Color of head, thorax, and abdomen black, red limited to narrow apical margin of second tergite, the following parts yellow: mouth parts, clypeus, face, narrow inner orbit slightly above antennal socket, cheek anteriorly to one-third eye-height but posteriorly only to curve of occipital carina, a narrow portion of postgena adjacent to hypostomal carina central 0.9 of propleuron, lower posterior margin of pronotum, prepectal carina, small area near anterior end of mesosulcus, tegula, subtegular ridge, wing base, apical half of scutellum, postscutellum, narrow apical margins of third to fifth tergites, broad apical margins of sixth and following tergites. Antenna black, reddish brown apically and below. Four anterior legs red with yellow coxae and trochanters; hind coxa black; hind trochanter yellow; hind femur red with broad basal and apical black annuli; hind tibia and tarsus dark brown.

#### *Allotype*

Male; resembling the holotype except in the following details: length 7 mm; yellow markings reduced; inner orbit black above antenna; gena and postgena completely black; subtegular ridge and lower parts of thorax completely black, only narrow apical margins of tergites three to six yellow, apical sternites and genitalia brown to dark brown.

#### *Egg*

Ovate, the surface not sculptured; stalk apical (Fig. 3).

#### *Variation*

*Female paratypes*.—Length 5 to 7.5 mm; cheek near lower margin of eye sometimes very weakly concave; sides of petiole usually diverging posteriorly. Postgena completely black in specimens from Vancouver Island; extent of yellow on thorax varying little but in darkest specimens (from Vancouver

Island) propleuron very extensively infuscated, prepectus completely black, and basal half of middle coxa black. In specimens from Oregon and Mt. Rainier, Washington, black of abdomen is more or less replaced by red; at the maximum extent tergites two to four completely red. Red of hind femur in smaller dark specimens reduced to lower central third of joint, the remainder black.

*Male paratypes*.—Length 5.5–8 mm. Extent of yellow on face varying from a small central triangle below antennal sockets to all of face except lower orbital triangles; front and middle coxae black basally in dark specimens; hind femur black in darkest specimens. In series of males from Mt. Rainier, ground color of abdomen varying from black, as in holotype, to largely red, with apical two-thirds of second tergite and all of third and fourth tergites red.

*Specimens Seen* (11 ♂♂, 15 ♀♀)

*Holotype*.—Female, Robson, British Columbia, June 6, 1949, H. R. Foxlee (CNC No. 6940).

*Allotype*.—Male, same data as holotype but June 20, 1945 (CNC).

*Paratypes*.—B.C.: 4 ♂♂, 3 ♀♀, Robson, May 28 to July 5, 1947 to 1950, H. R. Foxlee (CNC); 2 ♀♀, Goldstream, July 7, 1950, B. P. Beirne (CNC); ♀, Saanich district, Aug. 9, 1919, W. Downes (USNM). WASH.: ♀, Elbe, July 13, 1940, H. and M. Townes (Townes); 6 ♂♂, 6 ♀♀, Mt. Rainier, 2700 to 5500 ft, July 7 to 23, 1940, H. and M. Townes (Townes, USNM, and Swift). OREG.: ♀, Waldport, July 8, 1938, G. H. Townes (Townes).

This species occurs through a rather narrow area in the wet belts of the western Transition to Hudsonian zones. It flies in early and midsummer.

### ***Exyston (Exyston) Schiødte***

#### FLAVENS GROUP

This group contains only the Nearctic *flavens*. It is distinguished from other members of the subgenus by the pectinate tarsal claws, strongly convergent and strongly rounded cheeks (Fig. 20), and the short, stout habitus.

#### ***Exyston (Exyston) flavens* Davis**

*Exyston flavens* Davis, 1897, Trans. Am. Entomol. Soc. 24, 237 (o.d., ♀, type in ANSP).

This species is characterized by a short, stocky, and highly polished body with an abdomen broadly striped with yellow. The face is the broadest and the clypeus the smallest of any species in this genus (Fig. 19) and the cheeks in anterior aspect are the most strongly convergent (Fig. 20). It is the only Nearctic *Exyston* with pectinate tarsal claws.

#### *Female*

Length 6 mm; head not at all elongated; external apical margin of antennal scape shallowly and broadly excavated; face 2.4 times as wide as long (Fig. 19);

cheeks in frontal aspect converging at about  $90^\circ$ ; cheek strongly rounded; occipital carina strong and of uniform height, curving toward the hypostomal carina, and meeting it at an angle of about  $75^\circ$ .

Mesonotum with a very short but strongly elevated and open-ended ridge mesad of the lateral margin, submarginal groove deep and strongly foveolate in front of this; scutellum wider than long, shining and bearing only a few, small, sparse, punctures; propodeum fully carinated, short, and strongly declivous; apex of hind tibia with a small projection; tarsal claws pectinate.

Sides of petiole in dorsal aspect parallel; abdomen short and broad, second tergite as long as broad at apex; ovipositor valves not vomeriform, long and thin.

Color black, the following parts yellow: mouth parts, clypeus, face, inner orbit broadly to above top of eye, cheek, and temple nearly to top of eyes, postgena up to foramen magnum, propleuron, upper and lower margins of pronotum very broadly leaving only a narrow black saddle continuing irregularly back to first spiracle, prepectus, anterior third or more of mesopleuron and mesosternum, tegula, pair of dorsal discal lines on mesonotum, scutellum, postscutellum, a broad band extending from side to side across propodeum, apical third to half and sides of petiole, apical third of second and third tergites, apical half of fourth tergite and following tergites completely, venter of abdomen and genitalia except for first sternite. Antenna orange; lower half of scape yellow; remainder of scape, pedicel, and base of first flagellar segment black. Anterior and middle legs entirely yellow with some rufous infusion on the tarsi; hind coxa yellow, black basally and externally; trochanters yellow; femur red; tibia and tarsus reddish brown; extreme apex of femur and extreme base of tibia yellow.

#### *Male*

Unknown.

#### *Specimens Seen* (4 ♀)

*Specimens*.—MASS.: ♀, Milton, June 9, 1901, Percy Gardner, (MCZ); N.J.: ♀, Englewood, June 9, 1938, E. M. Greenspan (USNM); GA.: ♀, Rabun Bald Mountain in Rabun County, June 13, 1933, B. Dunavan, (Townes); TEX.: ♀, no other data (type) (ANSP).

The species is rare but probably occurs mainly in the Carolinian Zone. It flies in June.

#### VENUSTUS GROUP

The group contains the Nearctic *venustus*, *politus*, *exelsus*, *chamaeleon*, *humeralis*, and *illinois*. They are characterized by the apical projection on the hind tibia, submarginal lateral ridge on the mesonotum (Fig. 21), and the shape of the head. The cheeks are weakly convex, flat, or concave at the level of the lower end of the eye; they converge weakly in anterior aspect and are noticeably prolonged (Fig. 17).

***Exyston (Exyston) chamaeleon* n. sp.**

This species is characterized firstly by the uniformly black mesonotum and propodeum and secondly by the poorly developed mesonotal ridge, which is not really a ridge at all but simply a filled-in area in the usual site of the lateral foveolate groove of the mesonotum. The end of this ridge is not open, although an opening is sometimes weakly indicated.

***Holotype***

Female; length 8.5 mm; head somewhat elongated; external apical margin of antennal scape shallowly excavated; face about 1.95 times as wide as long, cheeks in frontal aspect converging at about 55°; cheek flat near lower margin of eye, making an abrupt angle of about 115° with postgena; occipital carina situated at corner of this angle, following edge of the rather narrow postgena to near hypostomal carina then curving abruptly toward hypostomal carina and becoming evanescent; cheeks strongly converging behind eyes at about 50°.

Lateral submarginal ridge of mesonotum low, not prominent, merely filling lateral groove posterior to middle of tegula, strongly declivous at posterior end but not open; scutellum slightly longer than broad, polished, and finely punctate; mesopleuron moderately densely punctate, but mesonotum polished and very sparsely punctate; propodeum completely carinate, not strongly declivous; apex of hind tibia with an externoventral projection.

Petiole in dorsal aspect slightly broadening throughout its length; ovipositor valves not vomeriform, pointed apically; ovipositor weakly sinuate.

Color of head and thorax black, the following parts yellow: mouth parts, clypeus, face, inner orbits to above top of eye, gena to middle of eye, postgena to foramen magnum, propleuron, pronotum very broadly below and irregularly above, lateral ridge of mesonotum, prepectus, anterior third of mesopleuron, mesosternum centrally, tegula, wing base, scutellum, postscutellum. Antenna orange; scape black above, yellow below; pedicel and basal two joints of flagellum black, remainder of flagellum black above basally. Four anterior legs yellow; their femora black behind basally, fading to red apically; hind coxa and trochanters yellow; hind femur yellow externally, red internally, with a basal black annulus and an apical yellow annulus; hind tibia yellow externally, red or brown internally, with extreme base yellowish and a subbasal black annulus; hind tarsus brown. Abdomen red; basal two-thirds of petiole black; a medium basal black spot on tergite four, tergite five black medio-basally, tergites six and seven black basally; large apicodorsal yellow spots on tergites one and two, much larger spot on tergite three and apical half of four and following tergites; lateral margins of third and following tergites yellow; venter, except for black first sternite, yellow; genitalia yellow.

***Allotype***

Male; resembling holotype except that cheeks are very weakly convex near lower margin of eye and not nearly so strongly convergent.

Color resembling that of holotype except in the usual greater extent of black, especially on the abdomen. Red color of abdomen confined to second,

third, and fourth tergites as follows: extreme base and apex of second tergite, third tergite except for extensive lateral and apical black infusion, fourth tergite mostly black but with some lateral and central red infusion. Yellow pattern differing from that of female as follows: black markings extending down face from antennal sockets; inner orbits extending only half way from antennal sockets to top of eye; gena and postgena black; pronotum, propleuron, mesopleuron, mesosternum, and mesonotum completely black; basal half of scutellum black; all coxae black basally, the hind coxa with only apical external half yellow. Apical yellow markings on abdominal tergites greatly reduced; the broadest not more than one-quarter length of tergite. Antennae generally darker than those of female.

#### *Variation*

*Female paratypes.*—Length 5 to 8 mm. Occipital carina usually evanescent before lower end of eye in more southern specimens, often so in northern ones; lateral ridge of mesonotum always flat-topped in southern specimens, often so in specimens from British Columbia and further north; ridge occasionally crossed by one or two carinae in southern and Prairie specimens; posterior end of ridge sometimes concave in specimens from British Columbia, thus appearing open-ended, but concavity not extending into ridge any further than width of ridge; costulae occasionally weak or incomplete; absent in one specimen. Yellow coloration often reduced, especially in specimens from Colorado and the Prairie Provinces and far northern areas. In the extreme reduction of yellow markings the pronotum is completely black; only anterior margin of mesopleuron and mesosternum yellow; hind coxa yellow only apically below; first and second tergites without yellow apical margins, and margins of other tergites reduced. Yellow pattern reaches its maximum extent in some specimens from British Columbia in which yellow spots appear on sides of propodeum. In many specimens, especially from Colorado, Oregon, Washington, and Saskatchewan, black replaces red on abdomen, third tergite and sides of fourth being last to change from red to black. Correlated with this change in color of abdomen is a change in dark markings of legs from red to black. Specimens from the far north and subarctic regions retain a red abdomen. The maximum extent of red is but little more than that of type, small red infusions appearing on the mesopleuron and sides of propodeum and metapleuron, and the first and fourth tergites becoming red. In blackest specimens antennal scape is completely black and antenna is brown, darker above.

*Male paratypes.*—The morphology varies as in the female but the hind tibial apical projection is rarely absent and the lateral ridge of the mesonotum is not so well developed, being sometimes almost absent and crossed by several ridges. In such cases the posterior declivity is always well marked. Posterior end of mesonotal ridge only occasionally concave. Color varies much as that of female, the abdomen varying from entirely black, especially in Colorado and the Prairie Provinces, to mainly red, with second, third, fourth, and sides of fifth tergites red, in some specimens from British Columbia

and the far north. Correlated with the greatest extension of red on the abdomen is a reduction of yellow markings, bands on apical tergites being reduced, narrow, and separated from yellow lateral margins; and first and second tergites having no yellow. The face varies from entirely yellow to mainly black with only lower orbital lines and a medium spot yellow. The usual black color pattern of the face is two lines originating near antennal bases and converging toward clypeus, where they often merge into a black clypeal suture. Dark markings of anterior and middle legs remain black in all specimens seen; hind coxa entirely black in darkest specimens but usually with a small yellow marking below or laterally.

*Specimens Seen* (32 ♂♂, 50 ♀♀)

*Holotype*.—Female, Robson, British Columbia, June 6, 1949, H. R. Foxlee (CNC No. 6944).

*Allotype*.—Male, same data as holotype but June 1, 1948 (CNC).

*Paratypes*.—Y.T.: Whitehorse, Firth River near Arctic Coast; N.W.T.: Aklavik, Fort Norman, Yellowknife, Alexander Falls on Hay River; B.C.: Robson, Osoyoos, Oliver, Summerland, Clinton, London Hill Mine at 7000 ft; WASH.: Mt. Rainier, Mt. Baker, Pullman; OREG.: Moiser, 5 miles west of Dufur, Kevin Ranch near Izee; CALIF.: Gold Lake in Sierra Co.; ARIZ.: Globe, Flagstaff; N.M.: Tajique; UTAH: Brightons; COLO.: near Estes Park, Granite Peaks at 9000 ft near Bayfield, North Park, Ft. Collins; S. DAK.: Capa; ALTA.: Bilby near Edmonton, Drumheller; SASK.: Saskatoon, Great Sandhills west of Swift Current, Roche Percee; ONT.: Smoky Falls on Matagami River.

This species has a wide range from the Hudsonian Zone to the Upper Sonoran Zone in the west and extends eastward across the prairies to Northern Ontario.

This species emerges from April or May in the south to July in the north and at high altitudes. The Arizona records for July and September perhaps are influenced by summer rains.

***Exyston (Exyston) excelsus* (Cresson)**

*Cteniscus excelsus* Cresson, 1865, Proc. Entomol. Soc. Phila. 4, 262 (o.d., ♀, Colorado, type in ANSP).

This species is very similar to *E. chamaeleon* n. sp. but is distinguished by the complete absence of red coloration, by the yellow dorsal vittae on the mesonotum of the female, and by the yellow markings on the propodeum of the male. It may be distinguished from *E. politus* Davis by the flat-topped mesonotal ridge, which is not open-ended.

*Female*

Length 8 mm, head somewhat elongated; external apical margin of antennal scape shallowly excavated; face about 1.95 times as wide as long, cheeks in frontal aspect converging at about 50°; cheek flat near lower margin of eye,

but making an abrupt angle of about  $110^\circ$  with postgena; occipital carina situated at the corner of this angle, disappearing near lower margin of eye, and never reaching hypostomal carina.

Mesonotum with a flat-topped ridge extending from about posterior end of tegula; this ridge strongly declivous at posterior end but never open-ended; scutellum slightly wider than long, highly polished, and very sparsely, finely, punctate; mesonotum and mesopleuron finely, but moderately, densely punctate, propodeum completely carinate with prominent costulae, somewhat rounded in profile; apex of hind tibia with a small blunt externoventral projection.

Petiole in dorsal aspect decidedly broadening throughout its length, apical width twice basal width; ovipositor valves not vomeriform, rounded apically, and straight on ventral side; ovipositor weakly sinuate.

Color black, the following parts yellow: mouth parts, clypeus, face, inner orbits broadly to above top of eyes, gena and postgena to level of middle of eyes and foramen magnum, sometimes broad inner orbits extending upward to meet upper orbits near top of eye, propleuron upper margin broadly and lower margin of pronotum, two dorsal vittae and lateral stripes on mesonotum—these four stripes sometimes prolonged forward along margins and notaulices to form a broad letter M—tegula, subtegular ridge, wing base, lower anterior half or third of mesopleuron, central half or more of mesosternum, prepectus with sternaulex remaining black, scutellum, postscutellum, sides of propodeum, sometimes a broad band extending across propodeum cephalad of apical carina, sometimes spots on metapleuron, apical third to half of first and second tergites, apical halves and lateral margins of third and following tergites, venter of abdomen except petiolar sternite and genitalia. Antenna orange, scape yellow below, upper part of scape, pedicel, first flagellar joint, and basal flagellar joints dorsally, black. Legs entirely yellow except for the following black parts: basal third of front femur internally, basal half of middle femur internally, hind femur internally, base of hind coxa internally, hind tibia internally; hind and middle tarsi brown.

#### *Male*

Resembling female except in the usual reduction of yellow markings. The yellow pattern differs as follows: inner orbits sometimes not extending above top of eye, gena and postgena black except immediately below the bottom of eye, propleuron black, pronotum black except for a few marginal spots in some individuals, mesosternum black except for yellow lateral ridges, prepectus and mesosternum black except for an anterior marginal spot, metasternum black, yellow of propodeum reduced to lateral spots, abdomen, antennae, and legs about as in female but yellow markings of abdomen slightly less extensive.

#### *Egg*

Ovate, stalk terminal.

*Specimens Seen* (2 ♂♂, 8 ♀♀)

*Specimens*.—COLO.: 3 ♀♀, 6 miles north of Boulder, June, 14–22, 1933 (Townes); 2 ♂♂, 4 ♀♀, foothills near Fort Collins, June and Aug., C. F. Baker (USNM and Townes); ♀, no further data (type) (ANSP).

This species has a very restricted range in the eastern foothills of the Rockies in Colorado.

***Exyston (Exyston) illinois* n. sp.**

This species is closely related to *Exyston humeralis* Davis. It may be distinguished by the occipital carina, which in *humeralis* is complete and of uniform height, but in this species is evanescent or absent at the lower end. In addition, the areola is shorter, with the sides most strongly convergent anteriorly, and frequently has a basal carina. This species is larger, more robust, and has a shorter and broader petiole than *humeralis*. In localities where they occur together *illinois* flies at least 2 weeks earlier.

***Holotype***

Female; length 8.5 mm; head elongated; external apical margin of antennal scape very shallowly excavated; face 1.8 times as wide as long; cheeks in frontal aspect converging at about 45°; cheek flat near bottom of eye, making an abrupt angle of about 100° with postgena; occipital carina situated at outer corner of this angle, not curving abruptly toward hypostomal carina but meeting it at an angle of about 45°, evanescent at its lower end.

Mesonotum highly polished and impunctate though hairy, with a prominent round-topped open-ended ridge extending back from about middle of tegula. Mesonotum and mesopleuron finely but moderately densely punctate. Propodeum completely carinate; areola with a strong, complete, basal carina; in profile not strongly declivous. Apex of hind tibia with a small blunt externoventral projection.

Petiole in dorsal aspect slightly broadening throughout its length; ovipositor valves not vomeriform, rounded apically; ovipositor weakly sinuate.

Color of head and thorax black, infused with red in center of mesonotum and sides of propodeum, the following parts yellow: mouth parts, clypeus, face, inner orbit to above top of eye, cheek to middle of eye, postgena to foramen magnum, propleuron, lower margin of pronotum, lower half of prepectus, lower anterior third of mesopleuron excepting sternaulex, central third of mesosternum, subtegular ridge, tegula, wing base, lateral mesonotal ridge, scutellum, postscutellum. Abdomen red, with basal halves of sixth and seventh tergites, petiolar sternite, and extreme base of petiole black, the following parts yellow: small apicodorsal spots on first and second tergites, central apical margin of third tergite, apical and broad lateral margin of fourth and fifth tergites, apical half of sixth and following tergites, venter of abdomen. Antenna orange with brown tip; scape, pedicel, and first flagellar segment, black; scape reddish below; remainder of antennal flagellum blackish dorsobasally. Four anterior coxae and trochanters yellow; remainder of

these legs reddish, but femora yellow anteriorly. Hind coxa yellow, black internobasally; hind trochanters yellow; hind femur red with basal brown annulus, extreme apex yellow; hind tibia and tarsus brown, darker dorsally.

*Allotype*

Male; resembling female except that basal carina of areola is absent and occipital carina curves more abruptly toward hypostoma carina, which it meets at a greater angle.

Color like that of female but with no red infusion on thorax. Yellow areas of head and thorax reduced as follows: postgena black, cheek yellow only anteriorly to one-third eye-height, propleuron black, pronotum, mesopleuron, and prepectus with reduced yellow areas, mesosternum completely black. Abdomen colored as that of female, except that basal three-quarters of petiole is black, and black areas of sixth and seventh tergite are reduced and colored brown.

*Egg*

Ovate; stalk apical.

*Variation*

*Female paratypes*.—Length 7 to 9 mm; basal carina of areola often absent; occipital carina occasionally absent at lower end. Color of head and thorax often mostly red, but apex of propodeum, area around scutella, center of pronotum, and ocellar triangle always black. Frons, postocciput, parts of mesosternum, and lower part of metapleuron frequently remaining black. Yellow areas often extended as follows: upper margin of pronotum, lower half or more of pronotum, lower anterior half or more of mesopleuron, mesosternum and prepectus entirely, discal mark on mesonotum, sides of propodeum and metapleuron, four anterior legs entirely except femora behind, entire hind coxa, and hind tibia below. Black of apical abdominal tergites becoming red in palest specimens but second to fourth tergites sometimes with latero-basal black infusions.

*Male paratypes*.—Resembling allotype but differing as follows in color: each abdominal tergite usually infused basally with black, in extreme cases red of abdomen occurring only in small apicolateral areas of third to fifth tergites; red of all femora sometimes replaced by black; yellow of lower thoracic areas sometimes so reduced as to be almost completely absent; extent of yellow on abdomen and legs sometimes about equal to that of the female.

*Specimens Seen* (7 ♂♂, 8 ♀♀)

*Holotype*.—Female, Ottawa, Ontario, May 28, 1933, W. G. Matthewman (CNC No. 6945).

*Allotype*.—Male, Hull, Quebec, June 11, 1924, C. H. Curran (CNC).

*Paratypes*.—QUE.: ♂, Lanoraie, May 31, 1936, J. I. Beaulne (Townes). MICH.: ♂, ♀, Midland Co., May 15, 1938, R. R. Dreisbach (Townes). ILL.: ♂, ♀, Southern Ill., Robertson (USNM); ♂, Plainview, May 3, 1915

(Ill.); ♂, ♀, West Union, May 22, 1884 (Ill.); ♀, Marshall, May 24, 1884 (Ill.); ♀, Watseka, June 4, 1932, Frison and Mohr (Ill.); ♀, Urbana, June 15, 1940, W. V. Balduf (Townes). IOWA: ♂, Henry Co., May 17, 1949, J. C. Schaffner (CNC); ♀, Mt. Pleasant, May 21, 1932, Longkeeker (USNM).

This species flies in late spring and early summer, earlier than most species of the genus. It is commonest in the Midwest but its range extends also into the St. Lawrence Valley.

***Exyston (Exyston) humeralis* Davis**

*Exyston humeralis* Davis, 1897, Trans. Am. Entomol. Soc. 24, 239 (o.d., ♂, Canada, type in ANSP).

*Exyston articularis* Davis, 1897, Trans. Am. Entomol. Soc. 24, 239 (o.d., ♂, Montana, type in ANSP).

This species is characterized by the combination of a strongly developed, sharp-topped, open-ended mesonotal ridge (Fig. 21), convex cheeks, and a complete, uniformly elevated, occipital carina.

*Female*

Length 5 to 8 mm; head somewhat elongated; external apical margin of antennal scape shallowly and broadly excavated; face about 1.8 times as wide as long, cheeks in frontal aspect converging at about 55° to 60°; cheek distinctly convex near lower margin of eye, but making an abrupt angle of about 120° with the postgena; occipital carina situated at the corner of this angle; curving almost uniformly toward the hypostomal carina and of uniform height, joining it at an angle of about 60°; antenna weakly tapered toward apex.

Mesonotum with a very prominent, sharply topped, open-ended ridge extending from about middle of tegula; scutellum about as long as broad, highly polished, and very sparsely punctate; mesopleuron moderately densely punctate except in speculum, but mesonotum very shining and almost completely impunctate; propodeum completely carinate, rather rounded in profile; apex of hind tibia with a small, blunt, externoventral projection.

Petiole in dorsal aspect slightly broadening throughout its length or slightly bulging near apex; ovipositor valves not vomeriform, pointed apically and straight on ventral side; ovipositor weakly sinuate.

Color red, the ocellar triangle black, frons, and region around scutellum sometimes black, the following parts yellow: mouth parts, clypeus, face, inner orbit to top of eye, gena to half eye-height, postgena to foramen magnum, propleuron, lower margin of pronotum, tegula, wing base, subtegular ridge, prepectus, lower anterior parts of mesopleuron, mesosternum centrally, scutellum, postscutellum, occasionally side of propodeum, sometimes medio-dorsal apical spots on first and second tergites, apical margin of third tergite, apical half and lateral margins of fourth and succeeding tergites, venter of abdomen except petiolar sternite, genitalia. Antenna orange with a black tip; scape red, its lower half yellow, sometimes blackish above; pedicel, first

flagellar joint, and dorsal part of basal flagellar joints black. Four anterior legs yellow, femora red behind, middle tarsi brown, anterior tarsi and anterior and middle tibiae often brown or yellow. Hind coxa yellow, red internally; hind trochanters yellow; hind femur red, extreme apex usually yellow; hind tibia brown with a poorly indicated subbasal black annulus, sometimes yellow externally and at extreme base; hind tarsus brown.

#### Male

Resembling the female except in the slight reduction of yellow coloration and the substitution of black for red on the head and thorax. The yellow pattern differs as follows: yellow inner orbits not extending to top of eyes, cheek yellow only anteriorly to near bottom of eye; postgena completely black; propleuron black; yellow lower margin of pronotum often greatly reduced and sometimes absent; prepectus, mesopleuron, and propodeum completely black; scutellum rarely black anteriorly. Abdomen varying from black to red; entire abdomen rarely black; usually petiole and basal half of second tergite black, remainder of abdomen red. Specimens with melanic abdomen are all small and the black color appears as a gradual suffusion progressing forward from the posterior tergites. Abdomen marked with yellow as follows: central apical margins of third and following tergites narrowly, occasionally lateral margins of fourth and following tergites, venter except first sternite, genitalia. Legs and antenna marked as in the female except the hind coxa, which is yellow, with the base, internal surface, and upper parts largely black; hind coxa rarely almost entirely yellow; hind femur usually black basally; hind tarsus and tibia sometimes dark brown.

#### Egg

Ovate; stalk terminal.

#### Specimens Seen (27 ♂♂, 17 ♀♀)

*Specimens.*—MONT.: 2 ♂♂ (types of *E. articularis* Davis) (ANSP). SASK.: ♂, Christopher Lake, July 20, 1939, A. R. Brooks (CNC). S. DAK.: ♂, Brookings (ANSP). KANS.: ♂, Cherokee Co., May 15, 1940, R. H. Beamer (Kans.); ♀, Riley Co., May, F. Marlatt (USNM). MINN.: ♀, Traverse Co., O. W. Oestlund (Minn.); ♀, Marshall, June 19, 1922, C. E. Mickel (Minn.). CANADA: ♂, (type) (ANSP). ONT.: 17 ♂♂, 9 ♀♀, Ottawa, June 9 to July 25, 1941–54, W. R. M. Mason and G. S. Walley (CNC); ♀, Gravenhurst, July 6, 1918 (Ohio). QUE.: 4 ♂♂, ♀, Montreal, June 15–18, (CU, CNC); ♂, St. Johns, June 1, 1902 (CU); ♀, Rouville Co., July 1, 1901 (CU); ♀, St. Placide, July 12, 1931 (USNM). NFLD.: ♀, Aug. 1919 (MCZ).

This species occurs east of the Rocky Mountains and mainly in the Transition Zone. The long series from Ottawa were all taken in partly shaded edges of an abandoned pasture. It flies in early summer but later in the Canadian Zone.

***Exyston (Exyston) venustus* (Cresson)**

*Cteniscus venustus* Cresson, 1865, Proc. Entomol. Soc. Phila. 4, 263 (o.d., ♀, Colorado, type in ANSP)

This species and the following are characterized by the very highly polished and sparsely punctate body, the complete absence of costulae, and the infumated wings. It may be distinguished from *politus* by its straight ovipositor (curved up in *politus*), central egg stalk (apical in *politus*), less concave cheeks, usually complete occipital carina (incomplete in *politus*), apically tapered antennae, and less strongly developed auriculae.

***Female***

Length 5.5 to 8 mm; head elongated (Fig. 17); external apical margin of antennal scape broadly but shallowly excavated; face 1.95 times as wide as as long, cheeks in frontal aspect converging at about 55°; cheek slightly to moderately concave behind lower part of eye, making an abrupt angle of about 110° with the postgena (Fig. 18); occipital carina situated at the corner of this angle; in the types curving toward the hypostomal carina and of uniform height, joining it at an angle of about 60°; in other specimens occipital carina weakened or completely absent at its lower end but always extending at least to lower margin of eye; antennae tapered at both ends, about 1.5 times as wide in middle as at apex; shortest joints slightly longer than wide, flagellum 27- to 30-jointed.

Mesonotum with a prominent round-topped open-ended ridge extending from about the anterior end of the tegula; scutellum about as long as broad, highly polished, and very sparsely, minutely, punctate; mesonotum and mesopleuron very sparsely, or not at all, punctate, highly polished; propodeum highly polished, very finely and very sparsely punctate, basal carina absent; costulae completely absent, although second lateral areas often lie below level of first lateral areas; apex of hind tibia with a small, blunt, extero-ventral projection.

Petiole in dorsal aspect broadening throughout its length; ovipositor valves not vomeriform, pointed apically. Ovipositor long, thin, and sharply pointed, straight or very slightly, uniformly, decurved.

Color of head, thorax, and abdomen red, the following parts yellow: mouth parts, clypeus, face, inner orbit broadly to above top of eye, cheek and temple to about two-thirds eye-height, postgena to level of foramen magnum, propleuron, upper and lower margins of pronotum very broadly, sometimes united and leaving only a small dorsal saddle (upper pronotal margin of types red except humeral angle), tegula, wing base and costal vein, subtegular ridge, prepectus, from anterior half to all of mesosternum, from lower anterior third to two-thirds of mesopleuron; sides of mesonotum and usually two stripes along notaulices, these four stripes often uniting into a letter M; apical third to entire scutellum, usually most of metapleuron and usually a broad stripe across propodeum, usually apicomedial third or half of petiole, apical margin or usually apical third of second tergite with a median prolongation approaching base of tergite, apical margin to apical half of third tergite and

successively broader margins on following tergites, fourth and succeeding tergites almost entirely yellow except for the anterior glabrous overlapping portions, venter of abdomen except petiolar sternite, genitalia. Four anterior legs entirely yellow except apical joints of tarsi and sometimes inner side of femora; hind coxa yellow, occasionally red above; hind trochanters yellow; hind femur yellow, usually red on inner surface, sometimes entirely red; hind tibia red internally, yellow externally, sometimes with poorly indicated blackish apical and subbasal annuli. Antenna orange; scape, pedicel, and first flagellar joints black; scape and sometimes pedicel yellow below. Wings distinctly infumated except in the apical 0.2.

#### Male

Resembling the female except that the cheeks are sometimes flat and the occipital carina is frequently complete.

Color like that of female but with a smaller extent of yellow markings. All the four male specimens seen are colored black. The yellow pattern differs from that of the female as follows: postgena completely black; gena black, usually only anteriorly to about one-third the eye height; inner orbit sometimes not extending to top of eye; upper half or entire propleuron black; pronotum black, some times a spot or stripe on lower margin yellow; mesonotum usually with only two short dorsal vittae; prepectus black; lower anterior, or anterior third, or less, of mesopleuron yellow, sometimes yellow markings of mesopleuron reduced to only a small spot; mesosternum usually black, sometimes a small yellow area along the prepectal carina; metapleuron and propodeum completely black; yellow pattern on abdomen similar to that of female but in apical segments never more than apical half yellow; all coxae black at extreme base; anterior and middle femora never black internally; anterior tarsi red; middle and hind tarsi red or black; antennal flagellum brown, not orange, extensively black above basally.

#### Variation

One female from the Chilcotin district, British Columbia, is anomalous in having strongly concave cheeks. The usual red areas of the head, body, and legs are colored black except the third and fourth tergites centrally.

#### Egg

Reniform, surface smooth; stalk very short, attached centrally.

#### Specimens Seen (5 ♂♂, 8 ♀♀)

*Specimens.*—B.C.: ♀, Chilcotin district, May 29, 1929, E. R. Buckell (CNC). WASH.: ♂, Walla Walla, June 10, 1934, G. E. Bohart (Townes). UTAH: ♂, Little Granite Mountain in Tooele Co., June 8, 1953, on *Sphaeralcea*, H. E. Cott (Utah). COLO.: 2 ♀♀, no further data (types of *C. venustus* Cress.) (ANSP); ♀, 6 miles north of Boulder, June 22, 1933 (Townes). S. DAK.: ♂, 2 ♀♀, Dixon, June 17, 1933, H. C. Severin (Townes). SASK.: 2 ♂♂, 2 ♀♀, Roche Percee, July 4–8, 1927, E. and S. Criddle (CNC).

This species occurs in the upper part of the Upper Sonoran Zone and lower Transition Zone and flies in early summer.

***Exyston (Exyston) politus* Davis**

*Exyston politus* Davis, 1897, Trans. Am. Entomol. Soc. 24, 240 (o.d., ♀, Southern California, type in ANSP).

The type specimen is red and yellow; all others known to me are black and yellow. Davis placed the other three specimens of the type series under *excelsus* but I can find absolutely no differences except that of color. The species may be distinguished by the characters discussed under *venustus*.

*Female*

Length 8 mm; head elongated; external apical margin of antennal scape broadly but shallowly excavated; face 1.95 times as wide as long, cheeks in frontal aspect converging at about 55°; cheek strongly concave behind lower part of eye, making an abrupt angle of about 110° with the postgena; occipital carina situated at the corner of this angle, completely absent at its lower end but extending to about lower third of eye; antenna tapered at base only, about 1.2 times as wide in middle as at apex; shortest joint at least as wide as long, flagellum 25- or 26-jointed.

Mesonotum with a prominent open-ended round-topped ridge extending from about the anterior end of the tegula; scutellum about as long as broad, highly polished, and very sparsely, minutely punctate; mesonotum and mesopleuron very sparsely punctate, highly polished; propodeum highly polished, very finely and very sparsely punctate, basal carina absent, costulae completely absent, although second lateral areas often lie below level of first lateral areas; apex of hind tibia with a small, blunt, externoventral protection.

Petiole in dorsal aspect broadening throughout its length, the basal auriculae unusually large, sharp, and prominent; ovipositor valves not vomeriform, pointed apically. Ovipositor long, thin, and sharply pointed; apical 0.2 abruptly curved upward.

Color of head, thorax, and abdomen black or red, the following parts yellow: mouth parts, clypeus, face, inner orbit broadly to above top of eye, cheek and temple to about one-half eye-height, postgena to level of foramen magnum, propleuron, upper and lower margins of pronotum very broadly, tegula, wing base, costal vein, subtegular ridge, prepectus, mesosternum centrally, lower anterior two-thirds of mesopleuron, sides of mesonotum and stripes along notaulices uniting into a letter M, scutellum, a broad stripe across propodeum, apicomedial third or half of petiole, apical third of second tergite with a median prolongation approaching base, apical half of third and following tergites, venter of abdomen except petiolar sternite, genitalia. Four anterior legs entirely yellow except brownish apical joints of tarsi and usually inner sides of femora basally; hind coxa yellow, occasionally black internobasally; hind trochanters yellow; hind femur yellow, red, or black on inner surface; hind tibia red or black internally, yellow externally, with a

poorly indicated brownish apical and subbasal annuli. Antenna orange with a black tip; scape, pedicel, and first flagellar joint black, scape yellow below. Wings distinctly infumated except in the apical 0.2.

*Male*

Unknown.

*Egg*

Ovate, surface smooth, stalk apical.

*Specimens Seen* (6 ♀ ♀)

*Specimens*.—CALIF.: 4 ♀ ♀, So[uthern] Cal[ifornia], no further data (including holotype) (ANSP); WYO.: ♀, Lusk, July 14, 1937, R. H. Beamer (Kans.); MON.: ♀, no further data (ANSP).

This species probably occurs in the Upper Sonoran Zone.

#### CINCTULUS GROUP

The group includes only *clavatus* and the European *cinctulus*. They are strongly characterized by the peculiar development of the occipital carina (see key) and excision of the outer apical margin of the scape. They also differ from other species of the subgenus in lacking the apical projection of the hind tibia and the submarginal lateral ridge on the mesonotum.

***Exyston (Exyston) clavatus* Cresson**

*Cteniscus clavatus* Cresson, 1864, Proc. Entomol. Soc. Phila. 3, 284 (o.d., ♂, Del., type in ANSP).

*Cteniscus abdominalis* Cresson, 1865, Proc. Entomol. Soc. Phila. 4, 264 (o.d., ♀, Colo., type in ANSP).

*Mesoleptus maculosus* Provancher, 1875, Naturaliste can. 7, 114 (o.d., ♀, Cap Rouge, Que., type in MPQ).

*Exyston abdominalis* Ashmead, 1896, Trans. Am. Entomol. Soc. 23, 197 (o.d., ♂, Beverly, Mass., type in USNM).

*Rhimphalea erythrogaster* Viereck, 1917, Conn. State Geol. and Nat. Hist. Bull. 22, 296 (o.d., ♀, Branford, Conn.).

*Exyston cinctulus* (Grav.) Mason, 1951, in Hymenoptera of America North of Mexico, Synoptic Catalogue. Edited by Muesebeck, Krombein, and Townes. U.S. Dept. Agr., Agr. Monograph, No. 2, p. 231.

*Exyston cinctulus clavatus* (Cress.) Kerrich, 1952, Brit. Museum (Nat. Hist.) Entomol. Bull. 2, 375.

This species is very closely related to the European species *Exyston cinctulus* Gravenhorst, but it is a much less hairy species than *cinctulus* (especially on the head) and has much less strongly developed occipital and hypostomal carinae. Specimens of *clavatus* from the Puget Sound area are colored as European ones, but those from other parts of North America are more rufus and less melanic. In particular, females of *cinctulus* have black and yellow, or black, red, and yellow hind coxa (red and yellow in *clavatus*), and mostly

black thorax and petiole (more extensively yellow and often suffused with red in *clavatus*). Males of *cinctulus* are generally more melanic, especially on the abdomen and hind legs.

These two species may be distinguished from all other *Exyston* by the peculiar structure of the cephalic carinae and the deeply excised externoapical margin of the antennal scape. Kerrich (1952 (1)) and I (1951 (4)) have treated *cinctulus* and *clavatus* as one species but since discovering how very closely many undoubtedly distinct species of *Exyston* resemble one another I prefer to regard them as two species. Their ranges are very widely disjunct and have probably been so since the pliocene or earlier.

#### *Female*

Length 8 mm; head elongated; external apical margin of antennal scape deeply excavated; face 1.85 times as wide as long; cheeks in frontal aspect converging at about 55°; cheek flat but rounded immediately in front of occipital carina; occipital carina continuing straight toward posterior mandibular condyle and not at all bending toward hypostomal carina, strongly elevated and hairy at its lower end, its outer margin irregularly crenulate.

Mesonotum without any ridge mesad to lateral margin, submarginal groove deep and strongly carinate; ventral part of prepectal carina very tall and directed forward; scutellum very coarsely rugulopunctate, longer than wide; propodeum fully carinated; apex of hind tibia without any projection.

Petiole in dorsal aspect broadening to spiracles at midlength, its sides subparallel or bulging behind spiracles; ovipositor valves not vomeriform, pointed and slightly decurved; ovipositor weakly to strongly sinuate.

Color of head and thorax black, parts of propodeum, meso- and metapleura and pronotum sometimes suffused with red, the following parts yellow: mouth parts, clypeus, face, inner orbit nearly to top of eye, gena and postgena to one-third eye-height, propleuron, lower margin of pronotum laterally, prepectus and adjacent parts of mesopleuron, subtegular ridge, tegula, apical half or more of scutellum, postscutellum. Antennal scape black to red, usually red or yellow below; pedicel and first flagellar joint black; remainder of flagellum orange, basal joints black above. Anterior and middle coxae and trochanters yellow; femora red; remainder of these legs reddish yellow. Hind coxa red basally and internally, yellow externally and apically; trochanter yellow; femur red, extreme apex yellow, base and subapical dorsal spot black; tibia red-brown, darker above, extreme base yellow, subbasal annulus and apical quarter blackish; tarsus blackish. Abdomen red, basal third or, usually, less of petiole black; venter yellow; second and following tergites basally infused with yellow, the amount increasing caudally so that fifth and following tergites are mostly or entirely yellow.

#### *Male*

Resembling female except that apical tergites are not so deep.

Color like that of female but with more black and less red and yellow as follows: inner orbits above antennae and entire head behind eyes black; thorax with no red infusions, but completely black except tip of scutellum, tegula, and occasionally small spots on propleuron; antennal scape usually black but often bearing a small yellow spot below; hind coxa completely black except for small apical yellow spots; petiole and basal third or more of second tergite black; yellow infusion on abdominal tergites much reduced, occupying less than half of apical tergites; apical tergites especially, infused with black or dark brown basally, red areas being completely replaced in extreme cases.

*Specimens Seen* (about 180 ♂♂ and ♀♀)

*Holotype*.—♂, Delaware (ANSP).

*Other Specimens*.—B.C.: Mission City; WASH.: Blaine, Sumas; CALIF.: Mountain View in Santa Clara Co.; COLO.: Denver, Montraose, Ft. Collins, Pagosa Springs; MONT.: Fairview; MAN.: Winnipeg; N. DAK.: Tower City, Hamar; S. DAK.: Watertown; KANS.: Lawrence, Wellington; MO.: Cliff Cave; ILL.: Bryant; IOWA: Ames, Sioux City, Dickinson Co., Keokuk Co., Henry Co.; MINN.: Cushing, St. Paul, Loman, Marshall, Barnum, Grande Marais, Itasca State Park, Sedan, Hennepin Co., Aitkin Co., Carver Co., Ramsay Co., Mille Lacs Co., Kittson Co., Houston Co.; MICH.: Midland Co., East Lansing, Douglas Lake; ONT.: Toronto, Waubamic, Salines, Coniston, Timagami, Smoky Falls on Matagami River, Ottawa, Cornwall; QUE.: Laniel, Montreal, Laurentian Mountains, Knowlton, St. Hilaire; N.B.: Fredericton; N.S.: Smith's Cover, King's Co., Hillsboro, Truro; P.E.I.: Canadian National Park; MAINE: Bristol; VT.: Bennington, St. Alban's; MASS.: Petersham, Lexington; R.I.: Westerly; CONN.: East Hartford; N.Y.: Maspeth, Wells, Mt. Kisco, Caroline-Harford; N.J.: Ramsey; PA.: Hazleton, Moosic; VA.: Blacksburg.

This species occurs east of the continental divide from the southern Canadian Zone to the Carolinian Zone. It is widespread and abundant in the midwestern states but scarce in the Atlantic coastal states. A possibly isolated population occurs on the Pacific Coast.

Throughout most of its range the species flies in June, but occurs as early as April in the south and in July and August in the extreme north. There is no evidence of a second generation. I have taken the species only in tall grasses in unmowed pastures or roadsides.

### Unplaced Species

*Exyston contracta* Davis, 1897, Trans. Am. Entomol. Soc. 24, 238 (o.d., ♂, Nevada; type in ANSP).

*Exyston nigroscutum* Davis, 1897, Trans. Am. Entomol. Soc. 24, 237 (o.d., ♂, Colorado; type in ANSP).

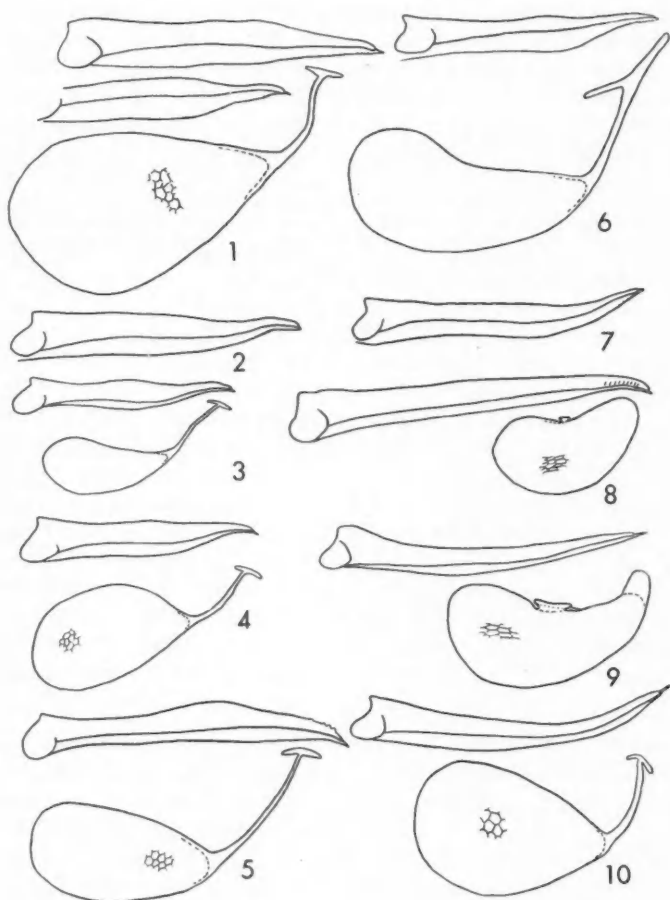
Both these species are founded on unique males. *E. contracta* is perhaps a synonym of *boreotis* but it could just as well fall in the *speciosus* group, belonging perhaps to the species I have called *spinulosus*. *E. nigroscutum* is identical

with several other odd males I have seen from Colorado to Alberta. Like the above, it may be a synonym of *boreotis* but could just as well belong to the *speciosus* group. Since I am often unable to place odd males in the *variatus* and *speciosus* groups I cannot definitely assign these two names to any species as defined here.

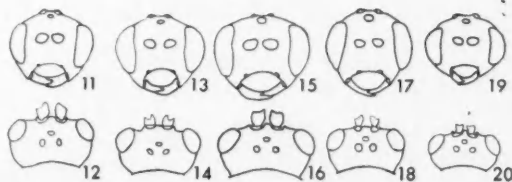
### References

1. KERRICH, G. J. A review, and a revision in greater part, of the Cteniscini of the Old World. Bull. Brit. Museum Entomol. **2**, 307-459 (1952).
2. MASON, W. R. M. A revision of the Nearctic Cteniscini (Hymenoptera:Ichneumonidae). I. *Eudiaborus* Kerrich and a new genus. Can. J. Zool. **33**, 18-73 (1955).
3. MASON, W. R. M. A revision of the Nearctic Cteniscini (Hymenoptera:Ichneumonidae). II. *Acrotomus* Hlgr. and *Smicroplectrus* Thom. Can. J. Zool. **34**, 120-151 (1956).
4. MASON, W. R. M. *Exyston cinctulus* (Grav.) Mason. In *Hymenoptera of America north of Mexico*, Synoptic Catalogue. Edited by Muesebeck, Krombein, and Townes. U.S. Dept. Agr., Agr. Monograph No. 2. 1951. p. 231.

NOTE: Figs. 1-22 follow.



FIGS. 1-10. Eggs and ovipositors of *Exyston* spp.  
FIG. 1. *Exyston variatus* Prov. FIG. 2. *E. hadros* n. sp. FIG. 3. *E. ater* n. sp. FIG. 4. *E. speciosus* Davis. FIG. 5. *E. spinulosus* n. sp. FIG. 6. *E. boreotis* Davis. FIG. 7. *E. austelli* n. sp. FIG. 8. *E. reniformis* n. sp. FIG. 9. *E. lophotos* n. sp. FIG. 10. *E. californicus* n. sp.



FIGS. 11-20. Dorsal and facial views of heads of *Exyston* spp.  
 FIGS. 11, 12. *E. tectus* n. sp. FIGS. 13, 14. *E. variatus* Prov. FIGS. 15, 16. *E. spinulosus* n. sp. FIGS. 17, 18. *E. venustus* (Cress.). FIGS. 19, 20. *E. flavens* Davis.

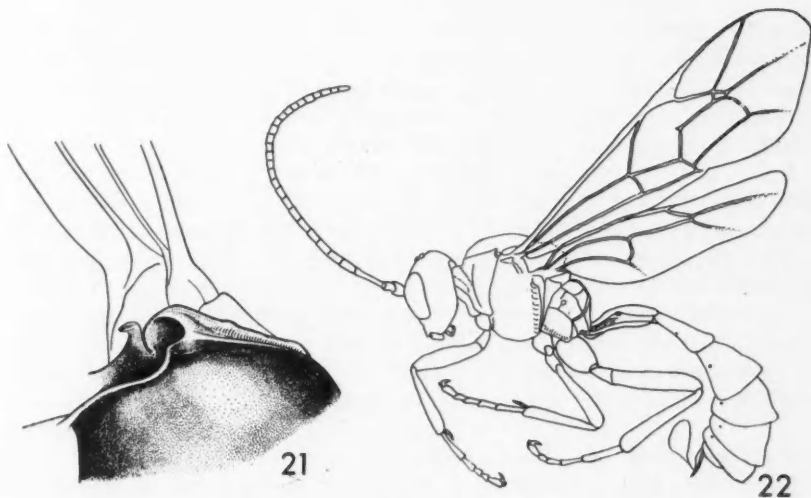
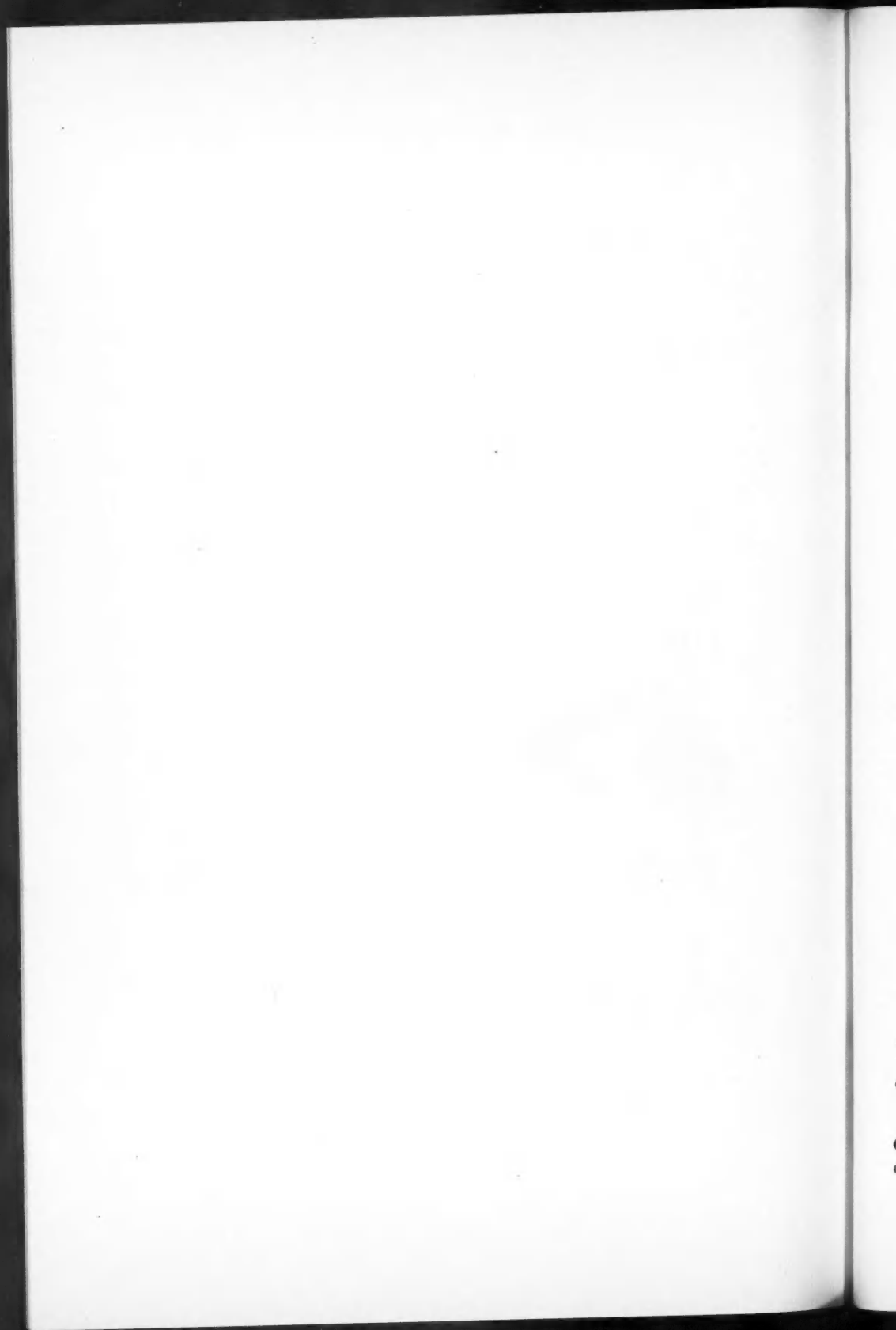


FIG. 21. *E. humeralis* Davis; left side of mesonotum showing base of wing, axillary tongue, mesonotal flange, and submarginal tent-like ridge characteristic of the *venustus* group. FIG. 22. Habitus sketch of *E. tectus* n. sp.



## MERASPID PERIOD (DEGREE 3) OF PSEUDOGYGITES LATIMARGINATUS (HALL)<sup>1</sup>

MADELEINE A. FRITZ

### Abstract

A specimen approximately 4 mm in length from the Craigeleith formation (Upper Middle Ordovician) on the shore of Georgian Bay near Craigeleith represents degree 3 of the Meraspid Period of the species *Pseudogygites latimarginatus* (Hall). The species is the characteristic index fossil found at the above horizon not only on Georgian Bay but elsewhere. Adult specimens occur in great abundance in southern Ontario, but no larval stages have been described. The present specimen is of special interest in that it furnishes significant information on the ontogeny of a widely known species.

It is assumed that trilobites, being Crustaceans, hatched from eggs. Minute ovoid bodies associated with certain species have been interpreted as eggs but the actual origin of these structures is not known. As trilobites ranged throughout the whole of the Palaeozoic era, and as they have been extinct since the Permian, it is little wonder that the tiny, fragile ova, even though initially buried, did not withstand the ravages of time.

Ever since the time of Barrande (1) growth stages of trilobites have been recognized despite the fact that examples of larvae are not common. To Barrande we owe much of our basic knowledge of the ontogeny of the group; he described and figured with great clarity developmental stages of several lower Palaeozoic species from Bohemia. These studies stimulated the research of later workers and subsequently successive stages were traced in a number of other species. Now three larval stages, following the egg, are known to occur, namely: the protaspis, meraspis, and holaspis. Raw (7) was the first to use this terminology.

The *protaspis* (*prot* primitive; *aspis* shield) was proposed by Beecher (2) for the simple, unjointed, horny, circular or ovoid disk, 1 mm (more or less) in diameter, which represents the beginning of the cephalon. The earliest protaspis may show only a slightly raised longitudinal axis but as development proceeds the animal molts and transverse grooves appear on the axis, denoting segments which are fused to form the cephalon. With later molts, postcephalic segments make an appearance. The protaspis period is considered to have ended with the formation of the primitive cephalon and the introduction of initial postcephalic segments, the latter regarded as the transitory or rudimentary pygidium.

The *meraspid* begins when the first trace of the actual pygidium is seen and when the first thoracic segment develops. With each molt an additional thoracic segment is introduced. At the same time there is an increase in over-all size. The number of meraspid molts corresponds to the number of

<sup>1</sup>Manuscript received June 1, 1959.

Contribution from the Department of Geological Sciences, University of Toronto, Toronto, Ontario.

thoracic segments of the adult; thus, if the species has eight thoracic segments, there will be eight meraspid molts termed "degrees". Each new thoracic segment was added from the anterior border of the pygidium. The actual growing points were at the posterior of the pygidium, where new segments were introduced; but free thoracic segments came in at the anterior margin of the pygidium.

The *holaspis* is the late larval stage during which no significant structures are introduced; the animal from this time on merely increases in size until adult stature is attained. The trilobite may continue to enlarge, with additional molts, until death occurs but there is no means of determining how many adult molts take place, as in the case of the meraspid.

The specimen which prompted me to prepare this paper was collected in the autumn of 1958 on one of our student field trips. Jane McNeill, a 'special' student in the Graduate School, spotted the tiny trilobite in the upper Middle Ordovician shales exposed on the shore of Georgian Bay near Craigeleith. These shales, now referred to as the Craigeleith formation, were formerly called Collingwood. The latter term is now used in the 'time' rather than 'formation' sense.

It is assumed that the larval specimen belongs to the species *Pseudogygites latimarginatus* (Hall) (4) better known to most palaeontologists as *Ogygites canadensis* (Chapman) (3). The taxonomy of the species is involved and is irrelevant to the present discussion, but an outline of the taxonomy will be given at the end of the description of the fossil.

Adult specimens of the above species (or fragments thereof) are exceedingly numerous in the Craigeleith shales; the species is the characteristic index fossil at that horizon both on Georgian Bay and elsewhere. Many of the specimens represent pygidia as well as other disarticulated skeletal parts. It is likely that many of the fragmentary remains represent molts; hence the number of individuals may not have been as abundant as it might seem. However, whole specimens are not uncommon. It may be, in the case of complete specimens, that the animal was present within the exoskeleton at the time of death. Whole adult specimens in our departmental collections at the University of Toronto vary from 20 mm to 128 mm in length, and all have eight thoracic segments.

#### *Specimen Described*

The larval specimen is well preserved and is practically complete. The dorsal side is exposed and the total length is 4 mm.

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#### PLATE I

FIG. 1. *Pseudogygites latimarginatus* (Hall), degree 3, Meraspid Period, magnified approximately 30 times. Surface untreated. Doublure markings and facial sutures well shown.

#### PLATE II

FIG. 2. *Pseudogygites latimarginatus* (Hall), degree 3, Meraspid Period, magnified approximately 30 times. Surface coated with magnesium oxide to bring out certain features, particularly the facets on the eye and the glabellar border with lateral and anterior ridges.

PLATE I

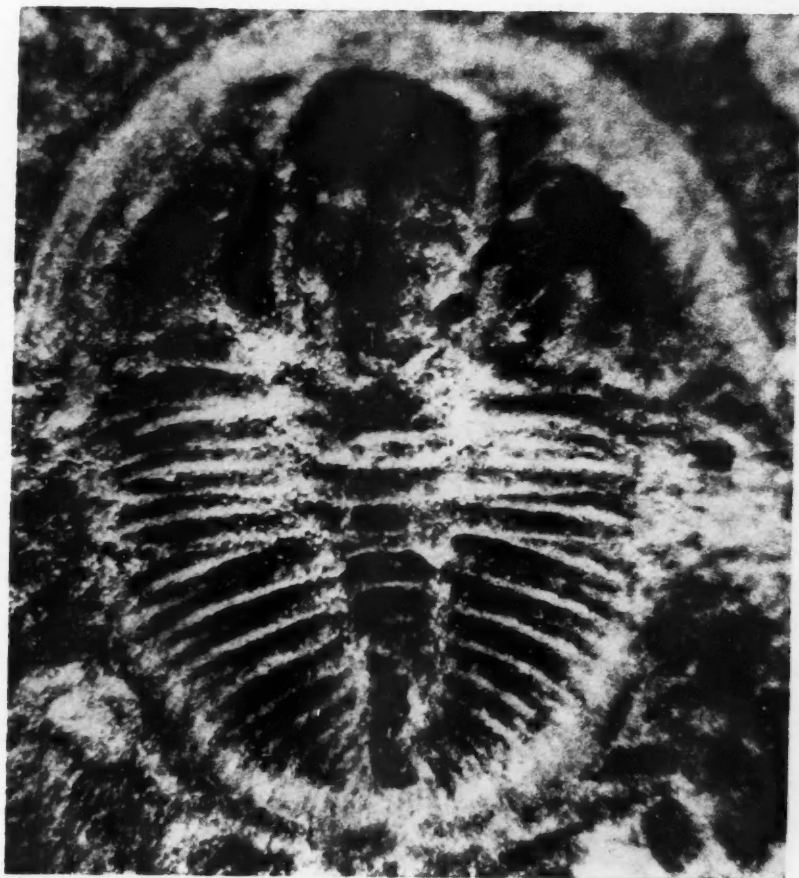




PLATE II





The *cephalon* is in the form of a broadly arched, horseshoe-shaped shield 2 mm long and 4 mm wide. It has a prominent, slightly concave border that maintains a constant width of almost 0.5 mm. Where the shell is broken away the doublure markings are well shown; they are coarser in relation to the size than in an adult. The border narrows gradually in its free extension, as genal spines, behind the head. The left spine may be traced as an impression as far back as the second thoracic segment but from the observed rate of taper it is assumed that the spines reached beyond the pygidium. The glabella is of low relief; it expands slightly anteriorly from the occipital ring and is rounded at its anterior end where it closely approximates the posterior margin of the cephalic border. Surrounding the glabella is a narrow depressed border (0.01 mm wide) with regularly spaced fine ridges crossing it at intervals about equivalent to the width of the border. The facial sutures are sufficiently well preserved to state that they extend forward and outward in advance of the eyes, then curve inward uniting on the cephalic border at a mid point in front of the glabella; posteriorly, the facial sutures, after encircling the palpebral lobes, extend at first obliquely and then downward to intersect the cephalon slightly behind the genal angles. The free cheeks are relatively large. The comparatively large crescent-shaped eyes are posteriorly situated on the cephalon, their distal ends being but slightly in advance of the occipital segment. They are located close to the glabella, thus restricting the area of the fixed cheeks. The left eye is well preserved with a length of 0.55 mm and a width of 0.3 mm. It exhibits 60 (or more) convex elevations quincuncially arranged. These elevations are interpreted as corneal lenses. The interareas at the base of the lenses are covered by very minute granules visible only under high magnification. In the adult of the species the compound eye appears to be smooth owing to the fact that the tiny lenses are covered by a glossy, chitinous integument, the cornea. If the convex elevations on this larva are corneal lenses it is assumed that the cornea was destroyed during fossilization, or that it had not been formed. However, larval 'instars' (intermolts) in many living Arthropoda often differ greatly from adults; for instance, nodes or tubercles, or other cutaneous structures, may be found on young shells that disappear entirely in the adult; or, the converse may be true. With this fact in mind it could be that this 'instar' possessed a cornea ornamented with surface granules that conceal the actual lenses lying beneath. A vertical section through the eye might clear up this debatable point but it is doubtful that a thin section could be prepared even at the expense of sacrificing the specimen. Packard (6) studied by means of thin section the eye of adult *Isotelus gigas*, a family relative of *Pseudogygites*. His sections, made from several specimens in which the eye was larger than the entire present larva, show both corneal lenses and cornea. Packard thus established the microscopic structure of this particular type of compound eye, known as 'holochroal'.

The *thorax* is 0.44 mm long and 2.5 mm wide. Three thoracic segments are present. The axis is relatively broad and shows a pronounced axial furrow; the pleural segments are deeply furrowed. As the adult of the species

has eight thoracic segments, it is clear that this animal had molted three times and that we are dealing with "degree 3" of the Meraspid Period.

The *pygidium* is 1.56 mm long; its maximum width is 2.5 mm. Both the axial rings and the pleural segments are very distinct in the anterior portion but they become fainter farther back where the last segments to form are situated. The presence of well-marked axial rings in the pygidium is clearly a youthful characteristic, adult forms show almost a smooth pygidial axis. The shape of the pygidium is semicircular whereas in the adult it tends to be pointed. A concave border is present as in the cephalon.

Table I gives comparative measurements in millimeters of three adults of varying sizes and the present larva.

TABLE I\*

| Total length | Cephalon |       | Thorax |       | Pygidium |       |
|--------------|----------|-------|--------|-------|----------|-------|
|              | Length   | Width | Length | Width | Length   | Width |
| Adults†      | 128      | 40    | 95     | 39    | 49       | 73    |
|              | 39       | 14    | 26     | 22    | 13       | 22    |
|              | 20       | 6     | 15     | 7     | 7        | 12    |
| Larva‡       | 4        | 2     | 4      | 0.44  | 2.5      | 1.56  |

\*All measurements in millimeters.

†Eight thoracic segments.

‡Three thoracic segments.

### Outline of the Taxonomy of *Pseudogygites latimarginatus* (Hall)

James Hall (4) described *Asaphus* (?) *latimarginatus* based on a pygidium from a small piece of shale found in the drift of New York State. Obviously no locality was available to supply topotypes and no head was present to provide diagnostic features.

Chapman (3) described from the shales at Whitby, Ontario, the species *Asaphus canadensis*. Chapman's description was inadequate, his types are unknown, and the locality from which his specimens were obtained is no longer available.

In the lack of other information it has always been assumed that '*latimarginatus*' and '*canadensis*' were identical and, on the basis of original descriptions, it would be difficult to prove otherwise.

Raymond (8) assigned '*canadensis*' to the genus *Ogygites*, which has facial sutures which meet in a point in front of the glabella instead of running around the anterior margin.

Kobayashi (5) erected a new genus, *Pseudogygites*, citing, as the type species, *Asaphus canadensis* Chapman. From his generic diagnosis Kobayashi must have had in mind the specimen described by Raymond from Ottawa as it is the only described specimen showing the features designated. *Pseudogygites* is characterized by a forked hypostoma whereas that structure in *Ogygites* is entire. Other differences have also been noted by Kobayashi.

Although the taxonomic 'tangle' is not yet settled current opinion holds that the Craigleith species should be referred to the species *Pseudogygites latimarginatus* (Hall).

For assistance with this taxonomy I am indebted to G. Winston Sinclair, Geological Survey, Ottawa.

### Conclusion

It is now over 100 years since the first studies in the ontogenies of trilobites were published. During the century, investigations by many scientific workers have added materially to Barrande's original pronouncements. Many more ontogenies, however, need to be worked out before the phylogeny of the Trilobita is understood and the taxonomy of the group established. As it is highly improbable that a complete suite of larval stages of a given species will be found, it follows that any new specimens that come to light should be carefully studied and the morphological features made known. The larval stage of *Pseudogygites latimarginatus* herein described has not been reported hitherto, hence this paper contributes information to the ontogeny of the Trilobita that has not been previously published. In that the development of this specimen follows the same pattern as that of various other species, this study serves to corroborate the views of previous workers with regard to the general developmental trends. In addition to the general larval features described, the specific character of the eye is of considerable significance. This particular eye structure does not seem to have been reported in previous studies.

The specimen is at present in the writer's collection, Department of Geological Sciences, University of Toronto.

### References

1. BARRANDE, J. Recherches paléontologiques, Trilobita. Système Silurien du centre de la Bohême. **1** (1852).
2. BEECHER, C. Larval stages of Trilobites. *Am. Geol.* **16**, 166-197 (1895).
3. CHAPMAN, E. J. *Can. J.* **1**, 482 (1856).
4. HALL, J. *Pal. New York*, **1**, 253 (1847).
5. KOBAYASHI, T. Faunal study of the Wanwanian (Basal Ordovician) series. *J. Fac. Sci. Imp. Univ. Tokyo, Sect. 2*, **3** (8), 461 (1934).
6. PACKARD, A. S. The structure of the eye of Trilobites. *Am. J. Sci.* **14**, 503-508 (1880).
7. RAW, F. The development of *Leptoplastus salteri* and other trilobites. *Quart. J. Geol. Soc.* **81** (2), 223-324 (1925).
8. RAYMOND, P. E. Description of some new Asaphidae. *Victoria Mem. Museum Bull.* **1**, 43 (1913).



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NOTES

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## LICE ON PHOCA HISPIDA SCHREBER

BETTY J. MYERS

Specimens of *Echinophthirus horridus* (Olfers) were collected from *Phoca hispida* Schreber (ringed seal) at South Baffin Islands by Dr. Ian McLaren of the Arctic Unit, Fisheries Research Board of Canada. This constitutes a new record although *E. horridus* has been recorded from other species of the genus *Phoca* and from other seals.

RECEIVED SEPTEMBER 19, 1959.  
INSTITUTE OF PARASITOLOGY,  
MCGILL UNIVERSITY,  
MACDONALD COLLEGE P.O., QUE., CANADA.

Can. J. Zool. Vol. 37 (1959)

## NOTE ON THE BLOOD OF DUCKS WITH LEUCOCYTOZOON DISEASE

A. SAVAGE AND J. M. ISA

In 1959 we briefly described an outbreak of *Leucocytozoon* disease that had occurred near Winnipeg during the previous summer (11). It wiped out several flocks of domestic ducks and provided us with blood smears showing various degrees of infection. A leisurely study of these has been undertaken because, at least until recently, there has been considerable disagreement as to the kind of cell that carries the mature gametocytes. Hence we note some of the observations and opinions that have been published concerning this point before considering the evidence itself.

When Danilewsky (2) described malaria-like parasites in the blood of birds and referred to them as *Leucocytozoaires*, he named a genus in the obvious belief that the infected cells were leucocytes.

Reservation as to the validity of this belief was expressed by Wenyon (12), who suggested that the cells concerned may have been erythrocytes. A more marked deviation was that of Hartman (4), who wrote, "there can be no doubt that the young parasites do enter red cells." His observations were confirmed by O'Roke (10) and by Fallis, Davies, and Vickers (3).

Outright heresy was the opinion of Martin (8), who is reported to have held that the attenuated forms of the organism take no part in gametogenesis. So far as we are aware, the reasons for his attitude have not been supported by other observers.

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On the other hand, Lapage (6) stoutly defended the original viewpoint by stating, without qualification, that the merozoites do not enter red cells but lymphocytes, monocytes, and macrophages.

Recently the conflicting viewpoints appear to have been harmonized by the discovery that the positive ones are well founded. Thus Huff (5) reported having found young gametocytes in cells of the colorless series and in late polychromatophile erythroblasts. However, a graded series of growth stages was seen only in lymphocytes, mononuclears, etc. He remarked, "While the cell infected with the fully grown gametocyte cannot be identified directly, the evidence indicates that it is a macrophage." Agreement was expressed by Fallis *et al.* (3) and by Morgan and Hawkins (9).

Evidence concerning the point at issue is therefore indirect. It includes a comparison of the differential leucocyte counts of infected birds with that of normal ones and noting of the differences, if any. With this in mind, we made differential counts in each of two films of blood from a heavily infected duckling. Giemsa's stain had been used. Gametocytes were classed as such. Recognizable lymphocytes and mononuclear cells showing early parasitism were put in their normal classes. The figures were in close agreement and the average result follows.

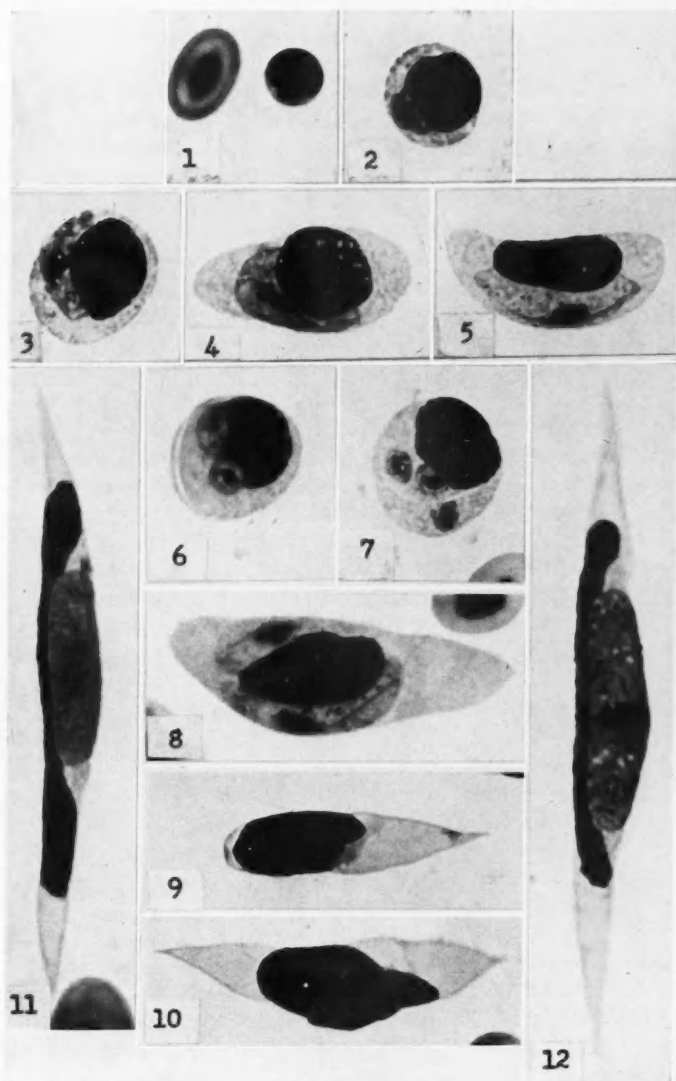
|              |         |
|--------------|---------|
| Lymphocytes  | 9.975%  |
| Polynuclears | 9.175   |
| Mononuclears | 0.805   |
| Basophiles   | 0.285   |
| (Subtotal)   | 20.22   |
| Gametocytes  | 79.74   |
|              | 99.96 % |

The blood picture of the normal duck has been determined by Magath and Higgins (7). Relevant figures from their publication are given in the first three columns of Table I. Column 4 shows the classes of cell entering into our subtotal above (20.22%) multiplied by five for direct proportional comparison. (It seems noteworthy that these figures all fall within the extremes given in columns 1 and 2.) From this it is evident that the lymphocytes and mononuclears are the varieties affected, the indication being that their decrease accounts for the otherwise unrecognizable forms classed as gametocytes.

TABLE I

A comparison of the proportions of the various classes of leucocytes in the blood of normal ducks with those of an advanced case of *Leucocytozoon* disease

|              | Normal |         |       | Infected |                   |
|--------------|--------|---------|-------|----------|-------------------|
|              | Lowest | Highest | Mean  | Observed | Diff. from col. 3 |
| Lymphocytes  | 45%    | 83%     | 61.7% | 49.87%   | -19.2%            |
| Polynuclears | 8.0    | 49.5    | 26.4  | 45.87    | +73.5%            |
| Mononuclears | 4.0    | 20.0    | 10.8  | 4.02     | -61.1%            |
| Basophiles   | 0      | 4.0     | 3.1   | 1.42     | -54 %             |



FIGS. 1-12. *Leucocytozoon simondi* (anatis).

1. Early invasion of lymphocyte. (Red cell included for size.)
2. Early invasion of monocyte.
- 3, 4, and 5. Normal developmental stages in monocytes.
6. Double invasion, monocyte.
7. Triple invasion, monocyte.
8. Advanced development of double invasion.
- 9 and 10. Abnormal gametocyte forms.
11. Mature normal microgametocyte.
12. Mature normal macrogametocyte.

Giemsa's stain; green filter; magn.  $\times 1350$ -1375.



The occurrence of multiple parasitism or hyperparasitism also might be introduced as indicative. Our observations showed the total leucocyte population to contain only 0.8% mononuclears recognizable as such. Of these, 63% were invaded, 45% by one merozoite, 18% by two. It is submitted that this last figure reflects a high degree of selectivity on the part of the invaders, especially since mature double gametocytes have not been recorded in ducks.

Here, perhaps, the findings of Coles (1) should not be ignored. He reported having found up to five immature leucocytozoa within a single host cell in the blood of English blackbirds. Unfortunately the degree of over-all infection was not stated, though we suspect it may have been severe. Hence comparison is impossible. For this reason and because the species concerned does not result in spindle-shaped forms, we merely note his observations. So far as ducks are concerned, in mild and moderate degrees of infection, we have not seen hyperparasitism of this kind.

A study of the recognizable but invaded leucocytes by classes is also helpful. Apparently no one has recorded having seen merozoites within polymorphonuclear leucocytes regardless of the staining quality of their cytoplasmic granules. Thrombocytes may be dismissed for the same reason.

The lymphocytes, however, deserve special consideration because of their variation in size. According to Magath and Higgins (7), the size varies from 4 to 8  $\mu$  but it is impossible to subdivide the cells into classes on that basis. Only 1.7% of those counted by us showed infection. There was no hyperparasitism. The appearance of the smaller ones strongly suggested the mechanical impossibility of their assuming the size and shape of typical gametocyte carriers. Whether or not the larger ones could do so is an open question (see Figs. 1-12).

This leaves the monocytes or macrophages as the most probable source of most gametocytes. That probability is increased by consideration of (1) their volume, (2) the hyperparasitism that only they reveal when infection is severe, and (3) a *residual* differential count as set forth above.

Except that we did not recognize parasitized cells of the erythrocyte series, our observations confirm those of Fallis *et al.* (3), Huff (5), and Morgan and Hawkins (9).

Finally, concerning the disease itself, it should be noted perhaps that when outbreaks come under attention, they are rather violent and have a high mortality rate. This may be because ducks are associated in flocks and blackflies in swarms—a pair of circumstances that makes for severe infection. As it is poor economics for a parasite to kill its host, we suggest that this may not happen as commonly to wild as to domestic birds. Of course, subclinical cases must occur but ordinarily they escape notice.

1. COLES, A. C. Blood parasites found in mammals, birds and fishes in England. *Parasitology*, 7, 17-61 (1914).
2. DANILEWSKY, V. Développement des parasites malairiques dans les leucocytes des oiseaux. *Ann. Inst. Pasteur*, 4, 427-431 (1890).
3. FALLIS, A. M., DAVIES, D. M., and VICKERS, M. A. Life history of *Leucocytozoon simondi* Mathis and Leger in natural and experimental infections and blood changes produced in the avian host. *Can. J. Zool.* 29, 305 (1951).

4. HARTMAN, E. The asexual cycle in *Leucocytozoon anatis*. *J. Parasitol.* **15**, 178-181 (1928).
5. HUFF, C. G. Schizogony and gametocyte development in *Leucocytozoon simondi*, and comparisons with *plasmodium* and *haemoproteus*. *J. Infectious Diseases*, **71**, 18-32 (1942).
6. LAPAGE, G. *Veterinary parasitology*. Oliver and Boyd, Ltd., London and Edinburgh. 1956.
7. MAGATH, J. B. and HIGGINS, G. M. The blood of the normal duck. *Folia Haematol.* **51**, 230-241 (1934).
8. MARTIN, R. K. The cytology of *Leucocytozoon anatis* Wickware. *J. Parasitol.* **19**, 179 (1932).
9. MORGAN, B. B. and HAWKINS, P. A. *Veterinary protozoology*. Burgess Publishing Co., Minneapolis, Minn. 1951.
10. O'ROKE, E. C. A malaria-like disease of ducks. *Univ. Mich. School of Forestry Bull.* No. 4 (1934).
11. SAVAGE, A. and ISA, J. M. A note on leucocytozoon disease in ducks. *Cornell Vet.* **49**, 252-254 (1959).
12. WENYON, C. M. Wellcome Trop. Research Labs. Third Rept. Khartoum. 1909. pp. 121-168.
13. WICKWARE, A. B. Is *Leucocytozoon anatis* the cause of a new disease in ducks? *Parasitology*, **8**, 17-21 (1915).

RECEIVED JULY 11, 1959.  
UNIVERSITY OF MANITOBA,  
WINNIPEG, MAN.

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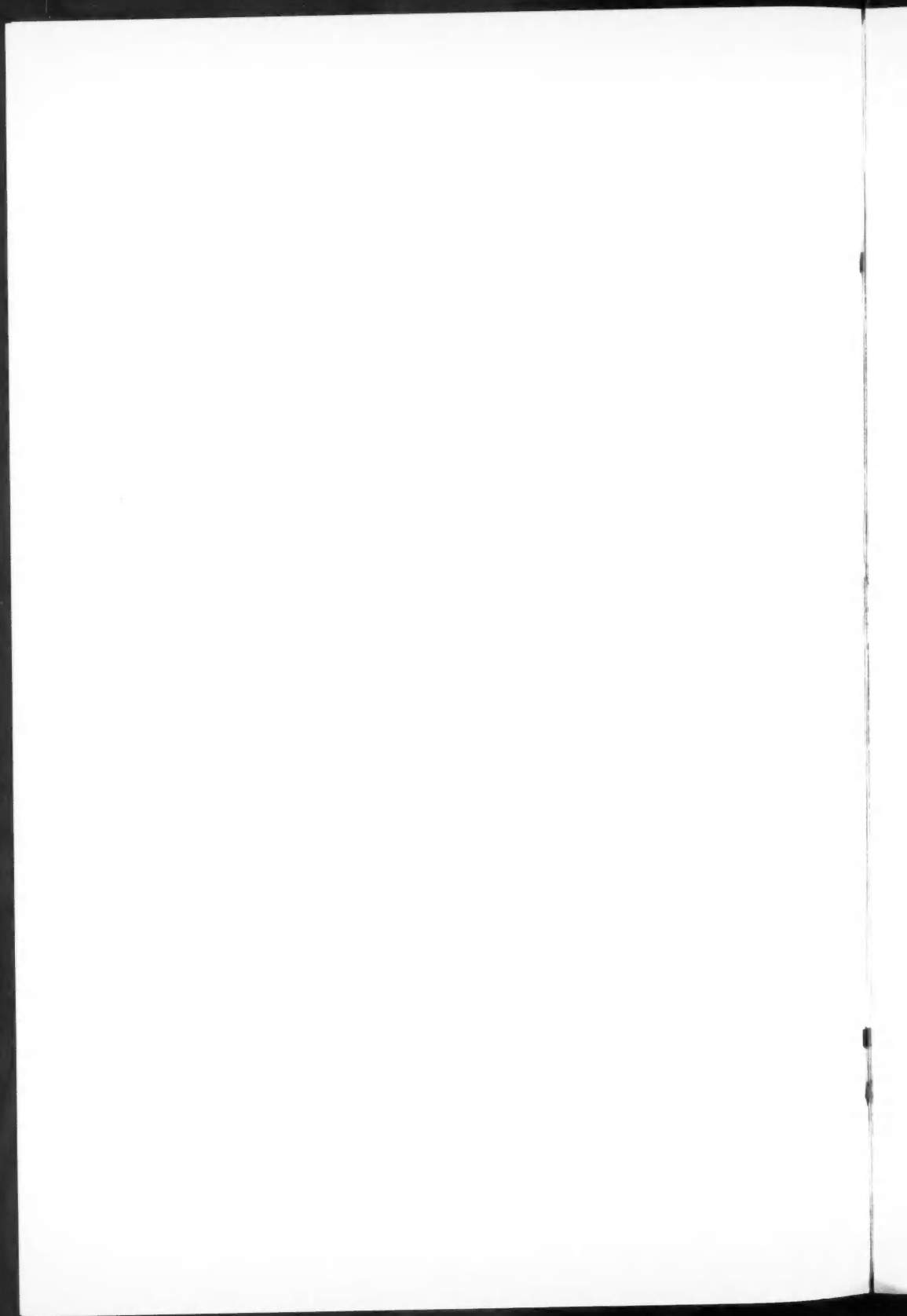
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Page 631. The caption for Fig. 1 should read "Time - percentage mortality curves for exposure to 45° C for liver-reared *P. affinis* larvae maintained at 23° C (●) and conditioned at 39° C for 2 hours (○)".

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Page 18. In line 21 of the section *Abdominal Process and Caudal Spine*, "anteriorly projecting spines" should read "posteriorly projecting spines".





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